AWARD NUMBER: W81XWH-17-2-0026

TITLE: Collaborative Research to Optimize Warfighter Nutrition (CROWN)III

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REPORT DATE: August 2020

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
August 2020	Annual	15 Jul 2019 – 14 Jul 2020

4. TITLE AND SUBTITLE

Collaborative Research to Optimize Warfighter Nutrition (CROWN)III

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-17-2-0026

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Louisiana State University System
Pennington Biomedical Research Center
6400 Perkins Rd
Baton Rouge, LA 70808-4124

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The Collaborative Research to Optimize Warfighter Nutrition (CROWN) III project is a series of 5 projects that use nutrition, metabolism and human physiology to address objectives relevant to the nutritional health of military personnel. Scientists at Pennington Biomedical Research Center and the Military Nutrition Division at the U.S. Army Research Institute of Environmental Medicine are collaborating on the design, execution and analysis of the 5 research projects that fall within three topic areas: Operational Stress and Nutritional Requirements; Nutrition, Inflammation and Resiliency; and Healthy Eating and Behavior. The 5 projects are as follows:

1. Determine the physiological and psychological effects of testosterone during severe energy deficit and recovery
2. Determine the substrate utilization, exercise performance, and skeletal muscle response to energy deficit and altitude acclimatization
3. Effects of energy balance and energy deficit on inflammatory response
4. Go for Green (GFG) Effectiveness
5. Development of a valid and military-appropriate survey tool for the assessment of Soldier eating behaviors

The outcomes will provide the scientific basis to develop novel nutritional programs, products and strategies that promote Warfighter health, performance and resilience to operational stress.

15. SUBJECT TERMS
Metabolism, nutrition, energy expenditure, readiness, performance, warfighter, resilience, operational stress

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	89	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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1. INTRODUCTION:

The Collaborative Research to Optimize Warfighter Nutrition (CROWN) III project is a series of projects that use nutrition, metabolism and human physiology research to address objectives relevant to the nutritional health of personnel in all branches of the military as well as the American public. Scientists at Pennington Biomedical Research Center and the Military Nutrition Division at the U.S. Army Research Institute of Environmental Medicine are collaborating on the design, execution and analysis of the research. The outcomes will provide the scientific basis to develop novel nutritional programs, products and strategies that promote Warfighter health, performance and resilience to operational stress. This project enables advances in our understanding of operational stressors on nutritional requirements; examines effectiveness of nutritional approaches for combating inflammation, sustaining gut health and promoting metabolic recovery; develops better tools for assessing eating behaviors; and establishes methods that lead to healthier food choices and overall diet quality. The body of work proposed by PBRC provides an efficient and cost-effective approach for achieving the DOD objective of a healthy and fit fighting troop base, ready for deployment and resilient to the stressors of duty. In order to achieve our objective, we are conducting a series of projects that fall within the following persisting gap (topic) areas:

1. Operational Stress and Nutritional Requirements
2. Nutrition, Inflammation and Resiliency
3. Healthy Eating and Behavior

We are conducting 5 projects that are listed below:

1. Determine the physiological and psychological effects of testosterone during severe energy deficit and recovery
2. Determine the substrate utilization, exercise performance, and skeletal muscle response to energy deficit and altitude acclimatization
3. Effects of energy balance and energy deficit on inflammatory response
4. Go for Green (GFG) Effectiveness
5. Development of a valid and military-appropriate survey tool for the assessment of Soldier eating behaviors.

2. KEYWORDS:

Metabolism, nutrition, energy expenditure, readiness, performance, warfighter, resilience, operational stress

3. ACCOMPLISHMENTS:

The major goals as defined in the statement of work involve 5 individual projects. Each of those projects is described below along with milestones, dates of completion, and current status.

Project 1: Determine the physiological and psychological effects of testosterone during severe energy deficit and recovery

Specific Aim: Clinical study to assess the effects of testosterone during severe energy deficit and recovery

Major Task 2: Data Analysis (months 6-24)

Data analysis for this project began in the second quarter of year 1 and is continuing.

Biospecimens (blood, urine, saliva, muscle tissue and fecal samples) were analyzed in years 1-2 for basic biochemistry markers, markers of endocrine function, gut hormones, inflammatory markers and measures of metabolism. Data analysis was completed and reviewed this year.

Analysis of questionnaires (including 3-day food records, cognitive tests and physical activity logs) is in progress.

All of the DXA body composition data has been compiled and is being analyzed. In total, there are 132 body composition variables that are measured and/or calculated during the DXA test. The primary outcome from DXA is lean body mass and fat mass and the distribution of these two compartments throughout the body. We held a conference call to discuss the details related to the variables and a manuscript is in progress.

Exercise and strength testing data from VO2 max and Biodex testing has been compiled and is in progress of analysis. The three main variables are strength, total endurance and peak torque.

Major Task 3: Data Interpretation and Publishing of Results

Milestone #1: Co-author initial manuscript on findings from clinical study (months 6-12)

The first manuscript was submitted for publication in the first quarter of year 2. It was reviewed, revised and resubmitted, and this milestone was met when the manuscript was published in the first quarter of this year. Additional manuscripts were published in the second and third quarters, and a fourth has been accepted for publication. Copies of the published manuscripts are included in the appendix.

1. Pasiakos SM, Berryman CE, Karl JP, Lieberman HR, Orr JS, Margolis LM, Caldwell JA, Young AJ, Montano MA, Evans WJ, Vartanian O, Carmichael OT, Gadde KM, Johannsen NM, Beyl RA, Harris MN, Rood JC. Effects of testosterone supplementation on body composition and lower-body muscle function during severe exercise- and diet-induced energy deficit: A proof-of-concept, single centre, randomised, double-blind, controlled trial. EBioMedicine. 2019 Aug;46:411-422. doi: 10.1016/j.ebiom.2019.07.059. Epub 2019 Jul 27. PMID: 31358477. PMCID: PMC6711889.

2. Hennigar SR, Berryman CE, Harris MN, Karl JP, Lieberman HR, McClung JP, Rood JC, Pasiakos SM. Testosterone Administration During Energy Deficit Suppresses Hepcidin and Increases Iron Availability for Erythropoiesis. J Clin Endocrinol Metab. 2020 Apr 1;105(4). doi: 10.1210/clinem/dgz316. Epub 2020 Jan 2. PMID: 31894236.

3. Karl JP, Berryman CE, Harris MN, Lieberman HR, Gadde KM, Rood JC, Pasiakos SM. Effects of testosterone supplementation on ghrelin and appetite during and after severe energy deficit in healthy men. J. Endocr. Soc. 2020 Apr 1;4(4):bvaa024. doi: 10.1210/jendso/bvaa024. eCollection 2020 Apr 1. PMID: 32258956. PMCID: PMC7101089.

4. Howard E, Margolis L, Berryman C, Lieberman H, Karl J, Young A, Montano M, Evans W, Rodriguez N, Johannsen N, Gadde K, Harris M, Rood J, Pasiakos S. Testosterone supplementation up-regulates androgen receptor expression and translational capacity during severe energy deficit. Am J Physiol Endocrinol Metab. July 2020 (Accepted)

Four abstracts also were presented at national conferences. Copies of the abstracts are included in the appendix.

1. Hennigar S, Berryman C, Harris M, McClung J, Rood J, Pasiakos S. Testosterone Administration During Energy Deficit Suppresses Hepcidin Concentrations and Increases Iron Utilization in Healthy Males. American Society for Nutrition, June 2019, Baltimore, MD. Curr Dev Nutr. 2019 Jun 13;3(Suppl 1). pii: nzz044.OR15-08-19. doi: 10.1093/cdn/nzz044.OR15-08-19. eCollection 2019 Jun. PMID: 31224847 PMCID: PMC6575034.

2. Howard EE, Margolis LM, Berryman CE, Lieberman HR, Karl JP, Young AJ, Montano MA, Evans WJ, Rodriguez NR, Johannsen NM, Gadde KM, Harris MN, Rood JC, Pasiakos SM. Testosterone supplementation upregulates myogenesis and attenuates proteolytic gene expression during severe energy deficit. American College of Sports Medicine, May 27, 2020.

3. Pasiakos S, Shankaran M, Berryman CE, Margolis LM, Lieberman HR, Karl JP, Young AJ, Montano MA, Matthews MD, Bizieff AA, Nyangau E, Mohammed HA, Harris MN, Evans WJ, Hellerstein MK, Rood JC. Effects of Testosterone on Mixed Muscle Protein Synthesis and Proteome Dynamics During Energy Deficit. American Society for Nutrition, May 2020.

4. Berryman C, Shankaran M, Nyangau E, Evans W, Hellerstein M, Rood J, Pasiakos S. Longitudinal Comparison of Lean Body Mass by Dual Energy X-Ray Absorptiometry and Muscle Mass by Creatine (methyl-d3) Dilution in Response to a 28-d Severe Energy Deficit. American Society for Nutrition, 29 May 2020. Current Developments in Nutrition, Volume 4, Issue Supplement_2, June 2020, Page 614, https://doi.org/10.1093/cdn/nzaa049_007.

The major finding in this trial was that subjects in the placebo group lost more body weight than those receiving testosterone. Fat mass decreased similarly in both groups while lean mass increased in the testosterone group compared to the placebo group. Bone mineral content did not differ between the two groups. Lower body muscle function declined in both groups and there was no preservation or improvement of muscle function in the testosterone group.

Hepcidin declined by 41%, indicators of iron turnover increased, and functional iron stores were reduced with testosterone administration during energy deficit. Testosterone administration during energy deficit increased circulating concentrations of erythropoietin and maintained erythropoiesis, as indicated by an attenuation in the decline in hemoglobin and hematocrit with placebo. Erythroferrone did not differ between groups, suggesting that the reduction in hepcidin with testosterone occurs through an erythroferrone-independent mechanism.

Testosterone supplementation during severe energy deficit prevented deficit-induced increases in fasting acyl ghrelin concentrations, and in post-prandial acyl and des-acyl ghrelin concentrations in nonobese, healthy men, but these effects did not blunt energy deficit-induced increases in appetite, slow recovery of total body mass, or impact changes in fat mass. These findings confirm and extend evidence supporting an inhibitory effect of testosterone supplementation on circulating ghrelin in healthy men, but suggest this effect is not sufficient to suppress appetite during energy deficit or subsequent body mass recovery.

Project 2: Determine the substrate utilization, exercise performance and skeletal muscle response to energy deficit and altitude acclimatization (study completed prior to award)

Specific Aim: Clinical study to assess the response to energy deficit and altitude acclimatization

This clinical study and data collection was completed under a previous award. However, some of the data is being analyzed and published under this award as Major Task 1. The timeline for Milestone #2, Co-author initial manuscript on findings from clinical study, was months 1-12 of this award. This milestone was met in year 1.

Two additional manuscripts were published this year as detailed in the Products section, and copies of the published articles are included in the appendix.

1. Bradbury KE, Berryman CE, Wilson MA, Luippold AJ, Kenefick RW, Young AJ, Pasiakos SM. Effects of carbohydrate supplementation on aerobic exercise performance during acute high altitude exposure and after 22 days of acclimatization and energy deficit. J Int Soc Sports Nutr. 2020 Jan 9;17(1):4. doi:10.1186/s12970-020-0335-2. PMID: 31918720. PMCID: PMC6953153.

2. Hennigar SR, Berryman CE, Kelley AM, Anderson BJ, Young AJ, McClung JP, Pasiakos SM. High-Altitude Acclimatization Suppresses Hepcidin Expression During Severe Energy Deficit. High Alt Med Biol. 2020 Apr 21. doi: 10.1089/ham.2019.0109. Epub ahead of print. PMID: 32316799.

This study examined the effects of consuming supplemental carbohydrate on aerobic exercise performance in recreationally active, healthy, young lowlanders at high altitude, before and following 22 days of acclimatization while in a constant state of negative energy balance. We demonstrated that consuming supplemental carbohydrate during steady-state exercise had no performance-enhancing effects on time trial performance before or after high altitude acclimatization. As expected, time trial performance was approximately 55% slower at acute high altitude compared to sea level. Acclimatization partially restored exercise performance, but performance at chronic high altitude was still 34% slower than at sea level. Carbohydrate supplementation had no effect on perceived effort during any of the performance trials. These data suggest that carbohydrate supplementation does not enhance aerobic exercise performance of lowlanders at high altitude.

Data also suggest that increased erythropoietic activity with high altitude acclimatization suppresses hepcidin expression to increase iron availability during severe energy deficit. The

decline in ferritin and increase in soluble transferrin receptor suggest a reduction in body iron stores with hypoxia and energy deficit even when dietary iron intake was held constant. The increase in transferrin saturation was a result of increased serum iron and total iron-binding capacity. Collectively, these data indicate that hypoxia is a strong signal for iron mobilization to support iron transfer to the erythropoietic compartment. Importantly, dietary iron and iron recycling from macrophages cannot match the increased requirements for iron at high altitude.

Project 3: Effects of energy balance and energy deficit on inflammatory response

Specific Aim: Clinical study to assess the effects of severe negative energy balance on inflammation, iron absorption, nutritional status, skeletal muscle and whole-body metabolic homeostasis, cognitive, and physical performance during a 96-h simulated sustained operations (SUSOPS).

Major Task 1: Conduct the clinical study (months 1-24)
In year 2, USARIEM investigators recruited, consented and enrolled 15 participants to participate in the study. In the first quarter of this year, 5 additional participants were recruited, consented and enrolled, and all biospecimens (blood and urine) were collected from the 3 completers (2 dropped) to complete this major task. The protocol required 15 subjects to complete testing to provide 80% power to detect significant differences in circulating hepcidin responses between SUSOPS 1 and SUSOPS 2.

Major Task 2: Data Analysis (months 24-48)
Blood and urine specimens were analyzed for basic biochemistry markers, markers of inflammation, gut hormones, markers of iron status and markers of energy expenditure. This year, we performed the following assays:

Test	Number of Samples
Zonulin	100
S100B	100
Pancreatic polypeptide	120
Osmolality	160
Lipoprotein Binding protein	100
GLP-1	120
Active ghrelin	120
Active PYY	120
I-FABP	80
Erythroferrone	350
IL-6	105
hsCRP	100
Erythropoietin	40
Hepcidin	105
Percent iron saturation	170
Total iron binding capacity	170
Iron	380
Ferritin	310

Soluble transferrin receptor	170
Erythroferrone	350
Leptin	80
Cortisol	100
Creatine kinase	100
DHEA-s	100
LDH	100
LH	100
Myoglobin	100
SHBG	100
Testosterone	100
BHBA	140
Free fatty acids	140
Glucose	140
Glycerol	140
Insulin	140
Sucralose	60
Erythritol	60

We also determined Total Daily Energy Expenditure and Total Body Water on 9 subjects at 2 time periods.

All data was transmitted to the Principal Investigator at USARIEM via a secure file transfer. Data analysis is complete and being reviewed.

Project 4: Go for Green (GFG) Effectiveness

Specific Aim: Determine the effectiveness of the Go for Green Program for changing dietary intake and attitudes toward nutrition for performance

Major Task 1: Conduct the clinical study (months 1-24)
In September 2019, 66 additional DFAC patrons were recruited, 27 consented and enrolled, and 6 completed the baseline assessments. Also, 26 additional DFAC staff participated in focus group sessions. Since the G4G project was closed on USARIEM's end, Catherine Champagne submitted closure documents to the PBRC IRB. G4G was officially closed at PBRC on April 7, 2020.

Major Task 2: Data Analysis (months 24-36)
All photo captures of Soldiers' trays have been completed. Estimation of tray contents was completed, and data was compiled and sent to the PI.

Major Task 3: Data Interpretation and Publishing of Results (months 36-48)
Discussions have continued regarding the drafting of manuscripts and abstracts. In addition to overall dietary intake and quality of the Soldier's diet, there is also interest in determining how specific food items changed due to the Go for Green concept. PBRC provided LTC Bukhari a comprehensive listing of food items used at both Forts Carson and Hood and subsequently

generated revised spreadsheets reflecting G4G colors and sodium content. This information was sent to USARIEM on March 31, 2020.

Two abstracts for Project 4 were presented at national conferences this year and are included in the appendix. A third abstract was accepted for the August 2020 Military Health System Research Symposium, which was subsequently cancelled; it will be resubmitted to the Association of Military Surgeons of the United States. Other presentations and a technical report/journal article are planned. LTC Bukhari, COL Cole, the G4G team, and Dr. Champagne meet twice monthly to discuss publications, abstracts and other deliverables from the G4G project.

1. Gideon S, Bustillos B, Armstrong NJ, Cole R. Military Dining Facility Staff Perspectives on the Implementation Barriers and Facilitators of Go for Green 2.0, a Department of Defense Nutrition Initiative. Society for Nutrition Education and Behavior Annual Conference, July 2019, Orlando, FL.

2. Champagne CM, Miketinas DC, Allen HR, Cole RE, McGraw SM, Moylan E, Armstrong N. Does Go For Green 2.0 Joint Service Performance-Nutrition Initiative Improve Dietary Intake of Soldiers? ObesityWeek, Nov 2019, Las Vegas, NV.

Diet quality (pre/post HEI scores, Mean ± SD) improved from 55.7 ± 9.5 to 64.1 ± 8.8, $p < 0.001$; attributed predominantly to 3 of 13 HEI constructs (3.6 pts whole grains, 1.1 pts seafood/plant proteins and 1.9 pts refined grains, all $p<0.05$). For patrons who consumed at least three meals in any combination during the study period (for example, breakfast with either two lunches or dinners), dietary fatty acids increased by 1.2 pts ($p=0.038$). More patrons agreed that main dishes were healthy/performance-based (32% pre; 57% post; $p<0.001$), vegetarian options were available (25% pre; 38% post; $p=0.023$), and they used nutrition labels to choose performance foods (38% pre; 54% post; $p=0.039$).

The G4G program successfully improved access to healthy/performance-based foods and improved the patrons' satisfaction and meal quality. The G4G program enabled patrons to select performance-based food choices. Strategies to overcome program barriers can further improve patrons' diet quality.

Project 5: Development of a valid and military-appropriate survey tool for the assessment of Soldier eating behaviors.

Specific Aim: Develop a military-specific eating behavior survey using input from subject matter experts and face-to-face interviews

Major Task 2: Validation of New Survey
Milestone #6: Development of the new/validated survey (months 24-36)
Validation of the new survey was completed this year. Ninety-eight subjects were enrolled in the study in February 2020 and data collection was completed in early July. Factor analysis was used to finalize the survey and this milestone was met in the fourth quarter.

Major Task 3, Data Interpretation and Publishing of Results
Milestone #7: Co-author initial manuscript on findings from clinical study (months 36-48)

This milestone was met when the initial manuscript was published this year. A second manuscript is in progress.

> Jayne JM, Ayala R, Karl JP, Deschamps BA, McGraw SM, O'Connor K, DiChiara AJ, Cole RE. Body Weight Status, Perceived Stress, and Emotional Eating among US Army Soldiers: A Mediator Model. Eat Behav. 2020 Jan;36:101367. doi: 10.1016/j.eatbeh.2020.101367. Epub 2020 Jan 16. PMID: 32018191.

Three abstracts using data from the study were presented this year and are included in the appendix.

1. Dyal RN, Deschamps BA, McGraw SM, Jayne JM, Karl JP, Cole RE. Using the Healthy Eating Score-5 as a measure of diet quality in an Active Duty Military population. Military Healthy System Research Symposium, August 2019, Kissimmee, FL.

2. Jayne JM, Ayala R, Karl JP, Deschamps BA, McGraw SM, O'Connor K, DiChiara AJ, Cole RE. Emotional Eating Behavior Mediates the Association between Perceived Stress and Body Weight Status. Military Healthy System Research Symposium, August 2019, Kissimmee, FL.

3. Stukenborg MJ, Deschamps BA, McGraw SM, Jayne JM, Karl JP, Cole RE. The association between military weight status and body image in a sample of active duty Soldiers. Military Healthy System Research Symposium, August 2019, Kissimmee, FL.

This study examined the relationship between perceived stress, body mass index (BMI), failing body composition (BC) standards and gender among US Army Soldiers to determine if emotional eating behaviors may mediate associations between perceived stress and outcomes of BMI and BC failure. Soldiers who reported emotional eating behaviors “sometimes” were nearly two times more likely to fail BC standards and were 3.25 pounds heavier (0.65 BMI points) than those who did not report emotional eating behaviors. Those who reported emotional eating behaviors “often” were two times more likely to fail BC standards and were 6.0 pounds heavier (1.19 BMI points) than those who did not report emotional eating behaviors. Although gender differences in the frequency of emotional eating behaviors were observed, they did not moderate the relationship with BMI or BC failure.

What opportunities for training and professional development has the project provided?
The data obtained from the Project 5 survey has been used for masters thesis projects for three MS/RD students enrolled in the Military-Baylor Graduate Program in Human Nutrition, and in a PhD dissertation for a US Army RD who obtained a PhD in Nutrition from Michigan State University.

How were the results disseminated to communities of interest?
Nothing to report

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

Project 1: Determine the physiological and psychological effects of testosterone during severe energy deficit and recovery

Major Task 2: Data Analysis will continue.
Major Task 3: Data Interpretation and Publishing of Results will continue.

Project 3: Effects of energy balance and energy deficit on inflammatory response

Major Task 3: Data Interpretation and Publishing of Results will continue.

Project 4: Go for Green (GFG) Effectiveness

Major Task 3: Data Interpretation and Publishing of Results will continue.

Project 5: Develop a military-specific eating behavior survey

Major Task 3: Data Interpretation and Publishing of Results will continue.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
Major findings from this project in the past year include the following:

Project 1
The major finding in this trial was that subjects in the placebo group lost more body weight than those receiving testosterone. Fat mass decreased similarly in both groups while lean mass increased in the testosterone group compared to the placebo group. Bone mineral content did not differ between the two groups. Lower body muscle function declined in both groups and there was no preservation or improvement of muscle function in the testosterone group.

Project 2
We demonstrated that consuming supplemental carbohydrate during steady-state exercise had no performance-enhancing effects on time trial performance before or after high altitude acclimatization. Acclimatization partially restored exercise performance, but performance at chronic high altitude was still 34% slower than at sea level. Carbohydrate supplementation had no effect on perceived effort during any of the performance trials. These data suggest that carbohydrate supplementation does not enhance aerobic exercise performance of lowlanders at high altitude.

Data also suggest that increased erythropoietic activity with high altitude acclimatization suppresses hepcidin expression to increase iron availability during severe energy deficit. Our data indicate that hypoxia is a strong signal for iron mobilization to support iron transfer to the erythropoietic compartment. Importantly, dietary iron and iron recycling from macrophages cannot match the increased requirements for iron at high altitude.

Project 4
The G4G program successfully improved access to healthy/performance-based foods and improved the patrons' satisfaction and meal quality. The G4G program enabled patrons to select performance-based food choices. Strategies to overcome program barriers can further improve patrons' diet quality. Military dining facilities are important avenues to improve diet quality and nutrition-related behaviors to optimize warfighters' fueling requirements.

Project 5
Soldiers who reported emotional eating behaviors "sometimes" were nearly two times more likely to fail BC standards and were 3.25 pounds heavier (0.65 BMI points) than those who did not report emotional eating behaviors. Those who reported emotional eating behaviors "often" were two times more likely to fail BC standards and were 6.0 pounds heavier (1.19 BMI points) than those who did not report emotional eating behaviors.

What was the impact on other disciplines?
Nothing to report

What was the impact on technology transfer?
Nothing to report

What was the impact on society beyond science and technology?
Nothing to report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

USARIEM investigators recruited, consented and enrolled 5 additional participants this year for Project 3. The protocol required 15 subjects to complete testing to provide 80% power to detect significant differences in circulating hepcidin responses between SUSOPS 1 and SUSOPS 2.

LTC Asma Bukhari replaced Nicholas Armstrong as Principal Investigator for Project 4: Go for Green (GFG) Effectiveness.

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

PBRC suspended on-site operations (including specimen analysis) from mid-March to mid-May due to the COVID-19 pandemic. Laboratory staff returned to work and resumed specimen analysis on May 18, 2020.

Changes that had a significant impact on expenditures
Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report

Significant changes in use or care of human subjects
Nothing to report

Significant changes in use or care of vertebrate animals
Nothing to report

Significant changes in use of biohazards and/or select agents
Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Three manuscripts for Project 1 were published this year and a fourth was accepted. Two additional manuscripts for Project 2 were published in Year 3. A manuscript for Project 5 also was published this year. Copies of the published manuscripts are included in the appendix.

1. Pasiakos SM, Berryman CE, Karl JP, Lieberman HR, Orr JS, Margolis LM, Caldwell JA, Young AJ, Montano MA, Evans WJ, Vartanian O, Carmichael OT, Gadde KM, Johannsen NM, Beyl RA, Harris MN, Rood JC. Effects of testosterone supplementation on body composition and lower-body muscle function during severe exercise- and diet-induced energy deficit: A proof-of-concept, single centre, randomised, double-blind, controlled trial. EBioMedicine. 2019 Aug;46:411-422. doi: 10.1016/j.ebiom.2019.07.059. Epub 2019 Jul 27. PMID: 31358477. PMCID: PMC6711889.

2. Hennigar SR, Berryman CE, Harris MN, Karl JP, Lieberman HR, McClung JP, Rood JC, Pasiakos SM. Testosterone Administration During Energy Deficit Suppresses Hepcidin and Increases Iron Availability for Erythropoiesis. J Clin Endocrinol Metab. 2020 Apr 1;105(4). doi: 10.1210/clinem/dgz316. Epub 2020 Jan 2. PMID: 31894236.

3. Bradbury KE, Berryman CE, Wilson MA, Luippold AJ, Kenefick RW, Young AJ, Pasiakos SM. Effects of carbohydrate supplementation on aerobic exercise performance during acute high altitude exposure and after 22 days of acclimatization and energy deficit. J Int Soc Sports Nutr. 2020 Jan 9;17(1):4. doi:10.1186/s12970-020-0335-2. PMID: 31918720. PMCID: PMC6953153.

4. Jayne JM, Ayala R, Karl JP, McGraw SM, O'Connor K, DiChiara AJ, Cole RE. Body Weight Status, Perceived Stress, and Emotional Eating among US Army Soldiers: A Mediator Model. Eating Behaviors. (In press)

5. Karl JP, Berryman CE, Harris MN, Lieberman HR, Gadde KM, Rood JC, Pasiakos SM. Effects of testosterone supplementation on ghrelin and appetite during and after severe energy deficit in healthy men. J. Endocr. Soc. 2020 Apr 1;4(4):bvaa024. doi: 10.1210/jendso/bvaa024. eCollection 2020 Apr 1. PMID: 32258956. PMCID: PMC7101089.

6. Hennigar SR, Berryman CE, Kelley AM, Anderson BJ, Young AJ, McClung JP, Pasiakos SM. High-Altitude Acclimatization Suppresses Hepcidin Expression During Severe Energy Deficit. High Alt Med Biol. 2020 Apr 21. doi: 10.1089/ham.2019.0109. Epub ahead of print. PMID: 32316799.

7. Howard E, Margolis L, Berryman C, Lieberman H, Karl J, Young A, Montano M, Evans W, Rodriguez N, Johannsen N, Gadde K, Harris M, Rood J, Pasiakos S. Testosterone supplementation up-regulates androgen receptor expression and translational capacity during severe energy deficit. Am J Physiol Endocrinol Metab. July 2020 (Accepted)

Books or other non-periodical, one-time publications.
Nothing to report

Other publications, conference papers and presentations.

Four abstracts for Project 1, two abstracts for Project 4 and three abstracts for Project 5 were presented at national conferences this year. Copies of the presented abstracts are included in the appendix.

1. Hennigar S, Berryman C, Harris M, McClung J, Rood J, Pasiakos S. Testosterone Administration During Energy Deficit Suppresses Hepcidin Concentrations and Increases Iron Utilization in Healthy Males. American Society for Nutrition, June 2019, Baltimore, MD. Curr Dev Nutr. 2019 Jun 13;3(Suppl 1). pii: nzz044.OR15-08-19. doi: 10.1093/cdn/nzz044.OR15-08-19. eCollection 2019 Jun. PMID: 31224847 PMCID: PMC6575034.

2. Gideon S, Bustillos B, Armstrong NJ, Cole R. Military Dining Facility Staff Perspectives on the Implementation Barriers and Facilitators of Go for Green 2.0, a Department of Defense Nutrition Initiative. Society for Nutrition Education and Behavior Annual Conference, July 2019, Orlando, FL.

3. Dyal RN, Deschamps BA, McGraw SM, Jayne JM, Karl JP, Cole RE. Using the Healthy Eating Score-5 as a measure of diet quality in an Active Duty Military population. Military Healthy System Research Symposium, August 2019, Kissimmee, FL.

4. Jayne JM, Ayala R, Karl JP, Deschamps BA, McGraw SM, O'Connor K, DiChiara AJ, Cole RE. Emotional Eating Behavior Mediates the Association between Perceived Stress and Body Weight Status. Military Healthy System Research Symposium, August 2019, Kissimmee, FL.

5. Stukenborg MJ, Deschamps BA, McGraw SM, Jayne JM, Karl JP, Cole RE. The association between military weight status and body image in a sample of active duty Soldiers. Military Healthy System Research Symposium, August 2019, Kissimmee, FL.

6. Champagne CM, Miketinas DC, Allen HR, Cole RE, McGraw SM, Moylan E, Armstrong N. Does Go For Green 2.0 Joint Service Performance-Nutrition Initiative Improve Dietary Intake of Soldiers? ObesityWeek, Nov 2019, Las Vegas, NV.

7. Howard EE, Margolis LM, Berryman CE, Lieberman HR, Karl JP, Young AJ, Montano MA, Evans WJ, Rodriguez NR, Johannsen NM, Gadde KM, Harris MN, Rood JC, Pasiakos SM. Testosterone supplementation upregulates myogenesis and attenuates proteolytic gene expression during severe energy deficit. American College of Sports Medicine, May 27, 2020.

8. Pasiakos S, Shankaran M, Berryman CE, Margolis LM, Lieberman HR, Karl JP, Young AJ, Montano MA, Matthews MD, Bizieff AA, Nyangau E, Mohammed HA, Harris MN, Evans WJ, Hellerstein MK, Rood JC. Effects of Testosterone on Mixed Muscle Protein Synthesis and Proteome Dynamics During Energy Deficit. American Society for Nutrition, May 2020.

9. Berryman C, Shankaran M, Nyangau E, Evans W, Hellerstein M, Rood J, Pasiakos S. Longitudinal Comparison of Lean Body Mass by Dual Energy X-Ray Absorptiometry and Muscle Mass by Creatine (methyl-d3) Dilution in Response to a 28-d Severe Energy Deficit. American Society for Nutrition, 29 May 2020. Current Developments in Nutrition, Volume 4, Issue Supplement_2, June 2020, Page 614, https://doi.org/10.1093/cdn/nzaa049_007.

- **Website(s) or other Internet site(s)**
  Nothing to report

- **Technologies or techniques**
  Nothing to report

- **Inventions, patent applications, and/or licenses**
  Nothing to report

- **Other Products**
  Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

The projects in this award are being carried out in collaboration with scientists from the U.S. Army Research Institute of Environmental Medicine; however, funds from this award are covering personnel costs at Pennington Biomedical Research Center only. I have detailed the contributions of Pennington Biomedical Research Center employees during this year:

Name: Jennifer Rood
Project Role: Principal Investigator
ORCID ID: 0000-0001-5848-2987
Nearest Person Month Worked: 7.2
Contribution to Project: She oversees/manages/coordinates the award and acts as the point of contact for interactions with USARIEM scientists and oversee all regulatory processes. She also serves as PI for project 1.

Name: Catherine Champagne
Project Role: Co-investigator
ORCID ID: 0000-0001-6127-1072
Nearest Person Month Worked: 2.7
Contribution to Project: She serves as the investigator for project 4.

Name: Ray Allen
Project Role: Computer support
Nearest Person Month Worked: 2.2
Contribution to Project: He provides computer support for project 4.

Name: Bridget Conner
Project Role: medical Technologist
Nearest Person Month Worked: 10.2
Contribution to Project: She provides analytical support in the clinical chemistry laboratory for projects 1, 2, and 3. She prepares specimen collection containers, processes specimens, analyzes biological samples, and reports results.

Name: Margaret Graves
Project Role: medical technologist
Nearest Person Month Worked: 6.0
Contribution to Project: She provides analytical support in the clinical chemistry laboratory for projects 1 and 3. She prepares specimen collection containers, processes specimens, analyzes biological samples, and reports results.

Name: Laura Holloway
Project Role: medical technologist
Nearest Person Month Worked: 6.0

Contribution to Project: She provides analytical support in the clinical chemistry laboratory for projects 1 and 3. She prepares specimen collection containers, processes specimens, analyzes biological samples, and reports results.

Name: Valery Hymel
Project Role: research associate
Nearest Person Month Worked: 10.2
Contribution to Project: She provides analytical support in the Mass Spectrometry Laboratory. She prepares specimen collection containers, processes specimens, analyzes biological samples, and reports results.

Name: Jessica Landry
Project Role: research specialist
Nearest Person Month Worked: 1.0
Contribution to Project: She provides analytical support in the clinical chemistry laboratory for project 3. She prepares specimen collection containers and processes specimens.

Name: Steve Lee
Project Role: medical technologist/lab manager
Nearest Person Month Worked: 10.2
Contribution to Project: He provides analytical support in the clinical chemistry laboratory for projects 1, 2, and 3. He prepares specimen collection containers, processes specimens, analyzes biological samples, and reports results.

Name: Stacey Roussel
Project Role: medical technologist/lab manager
Nearest Person Month Worked: 10.2
Contribution to Project: She provides analytical support in the clinical chemistry laboratory for projects 1, 2, and 3. She prepares specimen collection containers, processes specimens, analyzes biological samples, and reports results.

Name: Connor Sanford
Project Role: research project assistant
Nearest Person Month Worked: 11.0
Contribution to Project: He provides analytical support in the clinical chemistry laboratory for project 3. He prepares specimen collection containers and processes specimens.

Name: Jonathan Savoie
Project Role: research associate
Nearest Person Month Worked: 10.2
Contribution to Project: He provides analytical support in the Mass Spectrometry Laboratory. He prepares specimen collection containers, processes specimens, analyzes biological samples, and reports results.

Name: Tiffany Stewart
Project Role: Co-investigator

Nearest Person Month Worked: 1.8
Contribution to Project: She oversees project 5 and works on the development of a military specific eating behavior survey.

Name: Jamie Tuminello
Project Role: medical technologist
Nearest Person Month Worked: 12.0
Contribution to Project: She provides analytical support in the clinical chemistry laboratory for projects 1, 2, and 3. She prepares specimen collection containers, processes specimens, analyzes biological samples, and reports results.

Name: Dawn Turner
Project Role: research associate
Nearest Person Month Worked: 1.8
Contribution to Project: She provides visual estimation and food photography support for project 4.

Name: Edie White
Project Role: administrative assistant
Nearest Person Month Worked: 6
Contribution to Project: She provides administrative support for the entire award.

Total Expenditures to Date: $5,222,910.14

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

Nothing to report

What other organizations were involved as partners?

Organization Name: United States Army Research Institute of Environmental Medicine, Military Nutrition Division
Location of Organization: Natick, MA
Partner's contribution to the project: Collaboration (as detailed in the Statement of Work)

8. SPECIAL REPORTING REQUIREMENTS

QUAD CHART

Collaborative Research to Optimize Warfighter Nutrition (CROWN) III
Proposal Log #: JW160039
W81XWH-17-2-0026

PI: Jennifer Rood **Org:** Pennington Biomedical Research Center **Award Amount:** $6,665,000.00

Study/Product Aim(s)

The aim of CROWN III is to conduct research in nutrition, metabolism and human physiology to discover novel nutritional interventions, field feeding programs and food products that promote Warfighter resilience, improve Warfighter combat readiness and assure optimal Warfighter performance. Investigators from PBRC and USARIEM will collaborate on 5 projects:

1) Determine the role of testosterone on physiological and psychological factors during energy deficit
2) Evaluate whether added dietary protein can sustain lean body mass when underfed and operating at high altitude
3) Assess the effects of energy balance and energy deficit during sustained operations on inflammation and cognitive and physical performance
4) Quantify the effectiveness of the military dining facility intervention Go For Green on Soldier food choices
5) Develop a valid and military appropriate survey tool for assessment of eating behavior

Accomplishment: This project has provided the scientific basis to develop novel nutritional programs, products and strategies to promote Warfighter health, performance and resilience to operational stress.

Timeline and Cost

Activities FY	17	18	19	20
Protocol Development/ Approval				
Data Analysis				
Publication of Results				
Budget (M)	$2.165	$1.5	$1.5	$1.5

Updated: 8/14/2020

Goals/Milestones:

FY17 – Completed the clinical study for project 1, published initial manuscript on findings from project 2, obtained regulatory approval for projects 3 and 4, developed a draft survey for project 5

FY18 – Continued data analysis and interpretation for project 1, published additional manuscripts for project 2, completed the clinical study for project 3, conducted the study and analyzed data for project 4, began survey validation and data interpretation for project 5

FY19 – Continued data analysis and interpretation for projects 1 and 3, validated survey for project 5, published findings from projects 1-5

FY20 – Publish additional findings from projects 1-5

Budget Expenditures to Date: $5,222,910.14

9. APPENDICES: see following pages

EBioMedicine 46 (2019) 411–422

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

EBioMedicine
Published by THE LANCET

Effects of testosterone supplementation on body composition and lower-body muscle function during severe exercise- and diet-induced energy deficit: A proof-of-concept, single centre, randomised, double-blind, controlled trial

Stefan M. Pasiakos [a,*,1], Claire E. Berryman [a,b,c,1], J. Philip Karl [a], Harris R. Lieberman [a], Jeb S. Orr [a], Lee M. Margolis [a,b], John A. Caldwell [a,b], Andrew J. Young [a,b], Monty A. Montano [d,e,f], William J. Evans [g,h], Oshin Vartanian [i], Owen T. Carmichael [j], Kishore M. Gadde [j], Neil M. Johannsen [j], Robbie A. Beyl [j], Melissa N. Harris [j], Jennifer C. Rood [j]

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[d] MyoSyntax Corporation, Worcester, MA, USA
[e] Harvard Medical School, Boston, MA, USA
[f] Brigham and Women's Hospital, Boston, MA, USA
[g] University of California at Berkeley, Berkeley, CA, USA
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ARTICLE INFO

Article history:
Received 3 June 2019
Received in revised form 23 July 2019
Accepted 23 July 2019
Available online 27 July 2019

Keywords:
Hypothalamic-pituitary-gonadal axis
Hypogonadism
Military operational stress
Semi-starvation
Anabolism
Muscle mass
Lower-body muscle function

ABSTRACT

Background: Severe energy deficits during military operations, produced by significant increases in exercise and limited dietary intake, result in conditions that degrade lean body mass and lower-body muscle function, which may be mediated by concomitant reductions in circulating testosterone.
Methods: We conducted a three-phase, proof-of-concept, single centre, randomised, double-blind, placebo-controlled trial (CinicalTrials.gov, NCT02734238) of non-obese men: 14-d run-in, free-living, eucaloric diet phase; 28-d live-in, 55% exercise- and diet-induced energy deficit phase with (200 mg testosterone enanthate per week, Testosterone, n = 24) or without (Placebo, n = 26) exogenous testosterone; and 14-d recovery, free-living, ad libitum diet phase. Body composition was the primary end point; secondary endpoints included lower-body muscle function and health-related biomarkers.
Findings: Following energy deficit, lean body mass increased in Testosterone and remained stable in Placebo, such that lean body mass significantly differed between groups [mean difference between groups (95% CI), 2.5 kg (3.3, 1.6); $P < .0001$]. Fat mass decreased similarly in both treatment groups [0.2 (−0.4, 0.7), $P = 1$]. Change in lean body mass was associated with change in total testosterone ($r = 0.71$, $P < .0001$). Supplemental testosterone had no effect on lower-body muscle function or health-related biomarkers.
Interpretation: Findings suggest that supplemental testosterone may increase lean body mass during short-term severe energy deficit in non-obese, young men, but it does not appear to attenuate lower-body functional decline.
Funding: Collaborative Research to Optimize Warfighter Nutrition projects I and II, Joint Program Committee-5, funded by the US Department of Defence.
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1. Introduction

US military personnel conducting strenuous training and combat operations commonly experience sustained periods of severe, unavoidable energy deficit, the effects of which resemble the pathophysiology of

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https://doi.org/10.1016/j.ebiom.2019.07.059
2352-3964/Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Research in context

Evidence before this study

PubMed was searched for clinical trials published in English up to August 1, 2018, using search terms that included "weight loss", "starvation", "testosterone", "hypogonadism", "young men", "non-obese", "energy deficit", "military", and "muscle mass" alone or in combination. There have been no studies that have assessed whether restoring testosterone minimizes the catabolic effects of severe exercise- and diet-induced energy deficit in non-obese, young men reflective of military personnel participating in strenuous military operations.

Added value of this study

This proof-of-concept, single centre, randomised, double-blind, placebo controlled trial is the first, to our knowledge, assessing the efficacy of supplemental testosterone on body composition, muscle function, and clinical health biomarkers in healthy, non-obese, young men exposed to 28 d of severe exercise- and diet-induced energy deficit representative of strenuous military training and combat operations. Our study was rigorously controlled, particularly during the 28 d intervention phase, as diet and physical activity were individualized and continuously supervised on the in-patient unit at the Pennington Biomedical Research Centre. Despite the 28 d severe exercise- and diet-induced energy deficit, individuals receiving weekly injections of supplemental testosterone experienced an increase in lean mass and an attenuation in total weight loss compared to controls, with no difference in lower-body muscle functional decline or clinical health biomarkers.

Implications of all available evidence

Short-term (4 wk) use of supplemental testosterone minimizes weight loss and promotes lean mass gain in non-obese, young men exposed to military-relevant exercise- and diet-induced energy deficit, although the lean mass gain did not translate to improvements in muscle function. These data support further exploration of various pharmacologic interventions to mitigate the loss of muscle experienced by military personnel during periods of unavoidable, severe energy deficit, particularly those that may also safely enhance performance.

semi-starvation [1–3]. Energy deficits produced during strenuous operations are due, in large part, to increases in aerobic-type physical activity and restricted dietary intake, which often result in conditions that degrade both lean body mass and lower-body muscle function [4]. Further, many military personnel experience these severe and unavoidable periods of exercise- and diet-induced energy deficit and associated loss of lean body mass repeatedly over their military career, raising concerns about the potential accumulated health effects of those energy deficits. The US military has sponsored considerable research to develop nutritional countermeasures to mitigate lean body mass loss under these conditions; however, dietary interventions, including those attempting to leverage the anabolic potential of increased protein intake, have been ineffective during severe energy deficit [5–7].

The extent to which lean body mass is lost in men during severe energy deficit may, in part, be attributable to reductions in testosterone. Energy deficits inhibit the hypothalamic-pituitary-gonadal axis by preventing luteinising hormone release and testosterone synthesis [8]. Reductions in testosterone are accompanied by muscle atrophy and decreased muscle strength [9]. Testosterone status is markedly degraded during sustained military operations that involve prolonged, strenuous physical work, reduced energy intake, altered sleep, and psychological stress. Most notably, Friedl et al. [1] demonstrated a 50–65% decline in testosterone in male Soldiers during the first 28 d of US Army Ranger School. Therefore, exogenous testosterone administration may be an effective biomedical strategy to counter declines in testosterone, lean body mass, and physical function that result from severe energy deficit.

In a recent study, supplementing obese men who had low testosterone concentrations (≤12.15 nmol/L) with exogenous testosterone during 56-wk of underfeeding resulted in significantly more fat mass and less lean body mass loss than those receiving placebo injections [10]. However, no study, to our knowledge, has attempted to induce testosterone decrements by replicating the stressors responsible for energy deficits observed during military operations or test the subsequent efficacy and safety of supplemental testosterone for maintaining lean body mass and lower-body muscle function in non-obese males exposed to those military-relevant stressors. The purpose of this study was to evaluate the effects of supplemental testosterone on body composition and lower-body muscle function in non-obese, young adult males, representative of US military personnel, exposed to severe exercise- and diet-induced energy deficit for 28 d. We hypothesized that the group receiving supplemental testosterone during energy deficit would lose more fat mass, less lean body mass, and an equivalent amount of total body mass and have attenuated lower-body muscle functional declines compared to placebo. In addition, we hypothesized that testosterone supplementation during the energy deficit, compared to placebo, would result in more lean body mass accretion and less fat mass gain during recovery from exercise- and diet-induced energy deficit [2,11].

2. Materials and methods

2.1. Participants

Participants were recruited from the Baton Rouge, LA community. Procedures regarding recruitment, randomisation, and follow-up of this study are provided in Fig. 1. Participants were healthy (no cardiometabolic disorders), physically active (≥2-d/wk aerobic and/or resistance exercise) men aged 18–39 y, with total testosterone concentrations within normal physiological range [10.4–34.7 nmol/L, based on clinical practice guidelines at the time the study was conceived [12]]. Participants met age-specific US Army body composition standards [13]. Participants with prostate-specific antigen concentrations > 3 μg/L, haematocrit > 50%, a positive urine drug screen, or reporting anabolic steroid, human growth hormone, or nutritional testosterone precursor-like supplement use within the past 6-mo were excluded. Additional participant inclusion and exclusion criteria as well as details of the recruitment process are published elsewhere [14]. This study was approved by the Pennington Biomedical Research Centre Institutional Review Board and the Human Research Protection Office of the US Army Medical Research and Materiel Command. A data safety monitoring board, consisting of a biostatistician, an exercise physiologist, a clinician, and a layperson, oversaw the safety of participants, monitored recruitment and adherence, and reviewed adverse events, proposed modifications, and reports of related studies as appropriate. The data safety monitoring board was blinded to the study treatments, received quarterly reports, met annually, and provided written documentation of their assessments and recommendations for study continuation. Extended details of the roles and responsibilities of the data and safety monitoring board are provided as Supplementary Material. Participants provided written informed consent. Participants were studied between April 12, 2016 and September 15, 2017. This study is registered at www.clinicaltrials.gov as NCT02734238.

2.2. Study design and intervention

The 3-phase, proof-of-concept, single centre, randomised, double-blind, placebo-controlled, Optimizing Performance for Soldiers trial was conducted at Pennington Biomedical Research Centre to assess

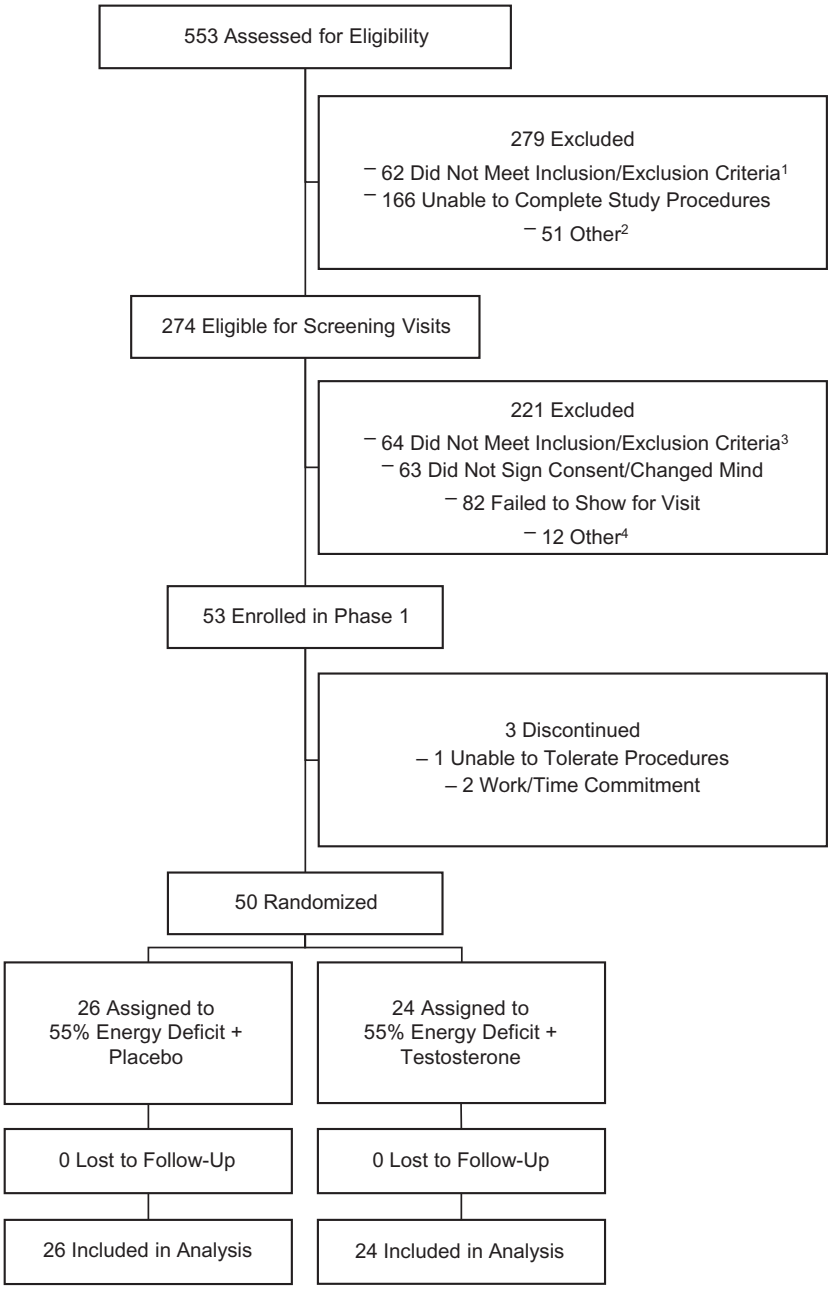

Fig. 1. Participant flow chart. 553 individuals were assessed for eligibility, 279 were excluded, 274 were found eligible for screening, 221 were excluded following screening, 53 were enrolled, 3 discontinued participation prior to phase 2 (i.e., were not randomised), 50 were randomised and completed the intervention (24 into Testosterone and 26 into Placebo). [1]Taking medications or supplements (n = 11), irremovable metal (n = 16), allergies or food intolerance (n = 5), not physically active (n = 2), age (n = 5), body mass index (n = 9), did not meet >1 inclusion/exclusion criteria (n = 14); [2]Recruitment complete/full (n = 28), could not contact (n = 17), not a citizen (n = 5), unknown (n = 1); [3]Medical history or lab results (n = 18), smoking/drug use (n = 12), body mass index (n = 20), dietary limitations (n = 3), non-compliant with screening procedures (n = 9), not willing to receive testosterone injections (n = 1), head circumference too large for MRI machine (n = 1); [4]Recruitment complete/full (n = 4), schedule conflict (n = 8).

the effects of exogenous testosterone administration on body composition changes during severe energy deficit and recovery (Fig. 2). Phase 1 was a 14-d (days 1–14), free-living, eucaloric diet period. Total daily energy expenditure for diet prescriptions during Phase 1 was determined using the Mifflin St. Jeor Equation with an activity factor of 1.3 to account for activities of daily living, and in combination with results from 7-d accelerometer data and 3-d activity logs collected during screening visits [14]. Diets provided 1.6 ± 0.2 g protein/kg/d distributed equally across meals, 30% of total daily energy requirements from fat, with the remaining energy derived from carbohydrate (Supplementary Table 1). Participants maintained their habitual (documented by pre-study, survey questionnaire) physical activity levels during Phase 1. Diet and physical activity adherence were verified by research dietitians, accelerometry, and by ensuring body mass was maintained within ±2%.

Participants were admitted to the inpatient unit after completing Phase 1 and underwent a 28-d (Phase 2; days 15–42), highly controlled exercise- and diet-induced energy deficit equal to 55% of Phase 2 total daily energy expenditure. This 55% energy deficit during Phase 2 involved increasing exercise-induced energy expenditure to elevate total daily energy expenditure by 50% above Phase 1 total daily energy expenditure, and restricting energy intake to 45% of this elevated total daily energy expenditure (Supplementary Table 2). The macronutrient composition of the Phase 2 diet was consistent with Phase 1. All meals were provided and consumed under supervision.

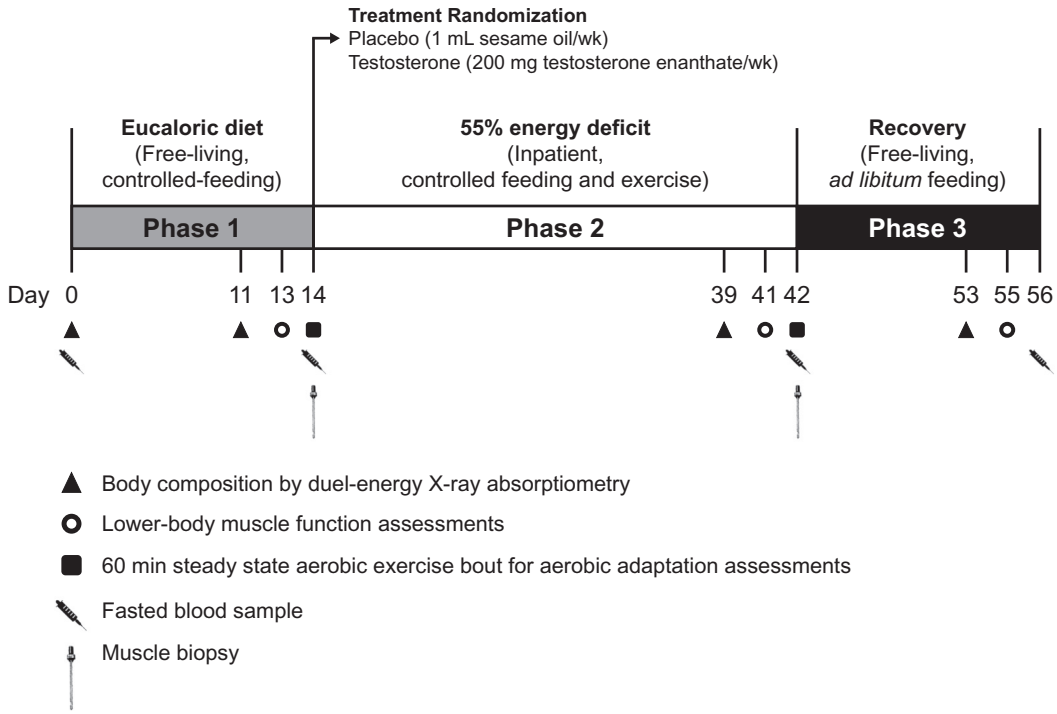

Fig. 2. Experimental design. Adapted from Pasiakos et al. [14].

To increase Phase 2 total daily energy expenditure, exercise consisted of varied, low-, moderate-, and high-intensity (40–85% of predetermined peak rate of oxygen uptake) aerobic-type exercise, including treadmill and/or outdoor walking and/or running, elliptical, stationary bike and weighted backpack (carrying 30% of body mass) walking (Supplementary Table 3). Steady-state, aerobic-type exercise was chosen as the primary means by which total daily energy expenditure was elevated during Phase 2 to reflect the aerobic-type physical work that occurs during sustained, strenuous military operations. High energy flux and limited dietary intake were the principal military-relevant stressors imposed in our study. Participants were not psychologically stressed, nor was sleep restricted in anyway during Phase 2. On average, participants performed 3.5 exercise sessions per day, all directly supervised by research staff. The physical activity prescription was verified biweekly and adjusted as needed to achieve the desired energy expenditure using open circuit indirect calorimetry (ParvoMedics TruOne 2400, East Sandy, UT). Light calisthenics were incorporated into the exercise routine every 3–4 d [14]. Adherence to the prescribed exercise was ≥89% and not different between groups (Supplementary Table 3).

After completing Phase 2, participants were released from the inpatient unit and instructed to return to their pre-study, ad libitum, habitual diet and physical activity routines; body mass and composition were assessed during this recovery period (Phase 3, days 43–56). Participants completed 3-d food records (1 weekend day, 2 weekdays) the week prior to day 56 that were reviewed by research dietitians.

2.3. Treatment randomisation and masking

At the start of Phase 2, participants were randomised according to a 1:1 ratio to receive either intramuscular injections of testosterone enanthate (200 mg/wk, Testosterone) or placebo (1 mL sesame oil, Placebo) on days 15, 21, 28, and 35 by a biostatistician unaffiliated with the study. The testosterone dosage was the same for each participant and chosen to maintain normal testosterone concentrations during severe energy deficit [14]. A computer-generated randomisation plan (SAS Institute, Cary, NC, USA, version 9.4) using the permuted-block method (n = 4/block) and age stratification (<29 or ≥29 y) was developed to allocate participants. The randomisation plan was delivered via a secured network to the study pharmacist who prepared the testosterone or placebo injections. The pharmacist was the only individual affiliated with the study that had access to the randomisation plan. The plan was kept in a password-protected file and accessed only when needed to allocate new participants to a group. Participants and all remaining study staff were blinded to group allocation until study completion.

2.4. Outcome measures

Body composition (total body mass, lean body mass, fat mass, and bone mineral content) measured on days 0, 11, 39, and 53 after an overnight fast and morning void by dual-energy X-ray absorptiometry (DXA; Lunar iDXA, GE Healthcare, Madison, WI, extended details on scanner positioning standards are provided as Supplementary Materials), was the primary study end point [14]. In this study, lean body mass is defined as total body mass minus fat mass and bone mineral content. All other outcomes described are considered secondary end points.

Semi-nude body weight was measured after an overnight fast and morning void by calibrated digital scale (GSE Inc. Model 450, GSE Scale Systems, Novi, MI) during each screening visit and daily throughout Phase 1 and Phase 2. During Phase 3, participants weighed themselves daily (semi-nude, after an overnight fast) using a calibrated scale provided by staff (Body Trace, Inc. Model BT003, New York, NY).

Percutaneous muscle biopsies of the vastus lateralis were collected in the rested, fasted state under local anaesthesia (1% lidocaine) using a 5 mm Bergstrom needle with manual suction on days 14 and 42 [15]. Muscle biopsy samples were used to characterize cross-sectional area and percent distribution of myosin heavy chain myofibres delineated as type II fast-twitch and type I slow-twitch at the end of Phase 1 and Phase 2. Muscle biopsy samples were also used to assess nuclear peroxisomal proliferator–activated receptor γ coactivator 1α percentage. Extended methodological details for these assessments are provided as Supplementary Material.

Lower-body muscular strength and endurance were assessed using isometric and isokinetic knee extension tests (Biodex Medical Systems, Shirley, NY) on days 13, 41, and 55. Knee extensor dynamometry was chosen to assess muscle function due to its reliability in assessing muscle strength in response to changes in lean mass [16]. Isometric peak torque was measured at 75° knee flexion (3 repetitions, separated by 30 s rest). Isokinetic peak torque was determined from six maximal knee extensions at 60° per second, and isokinetic total work was determined from 20 repeated maximal knee extensions at 180° per second. Muscle strength and endurance data are presented as absolute values and relative to lean body mass of the tested leg.

Participants also performed 60 min steady-state aerobic exercise bouts on days 14 and 42 to characterize submaximal aerobic adaptations (e.g., heart rate, ratings of perceived exertion, power output, energy expenditure, and substrate oxidation) to testosterone and severe energy deficit. Exercise intensity was matched within participants based on power output and total work performed to limit the confounding effects of weight loss on relative exercise intensity [14].

2.5. Sample analyses

Fasted blood samples were collected on days 0, 14, 42, and 56 between 06:00 h and 09:00 h to limit the potential confounding effects of circadian rhythm on total testosterone in young men [17]. Blood samples were analysed for total testosterone, follicle-stimulating hormone, estradiol, sex hormone-binding globulin, luteinising hormone, insulin, cortisol, and prostate-specific antigen (Siemens Immulite 2000, Llanberis, UK). Free testosterone was determined by calculation [18]. Insulin-like growth factor-1 was analysed using an enzyme-linked immunoassay (ALPCO, Salem, NH). Glucose, total cholesterol, HDL-cholesterol, triglycerides, and alanine aminotransferase were analysed on a Beckman DXC 600 Pro (Brea, CA). LDL-cholesterol was determined by calculation [19]. Complete blood counts were analysed on a Beckman DxH (Brea, CA). Systolic and diastolic blood pressure were measured manually on days 0, 15 (prior to the first treatment injection), and 42.

2.6. Sample size determination

Relevant data (means ± standard deviations) demonstrating the effects of moderate-to-severe energy deficit on lean body mass were used to determine statistical power and sample size [20,21]. Based on these estimates, with 90% statistical power, 5% type I error rate, and according to a 2-sided test, 22 males per group were needed (n = 44) [14]. Based on the variability in lean body mass loss in response to moderate-to-severe energy deficit, the sample size was increased to n = 50.

2.7. Data collection and management

Participants were assigned unique identification numbers that were used on all data collection forms, questionnaires, biological specimen tubes, and computer records. The master list linking participant names and identification numbers were kept in a password-protected computer file with access only granted to the principal investigator and study manager. Biological samples moved off-site for analysis (i.e., MyoSyntax performed the muscle cross-sectional area and nuclear peroxisomal proliferator–activated receptor γ coactivator 1α analyses) did not contain any personal identifiable information, nor did they have access to the master list at any time.

Data collection forms were kept under lock and key, or in password-protected files, and under the control of the principal investigator and project coordinator. The Pennington Biomedical Research Centre has a fully integrated, campus wide, automated Central Database management system that is fully validated and continually vetted for quality assurance. Data from self-report or questionnaires were also collected and managed using the REDCap electronic data capture tools hosted at the Pennington Biomedical Research Centre [22]. Data are backed up daily, and the Research Computing Core at the Pennington Biomedical Research Centre oversees all data management.

2.8. Statistical analysis

All analyses are considered 2-tailed, with $\alpha = .05$ considered statistically significant. All primary analyses were intention-to-treat. When luteinising hormone (n = 15), follicle stimulating hormone (n = 27), estradiol (n = 3), and prostate-specific antigen (n = 2) values were below the level of detection, values for data analyses were imputed at one quarter of the detection value. A single testosterone observation was above 55.5 nmol/L and was imputed as 55.5 nmol/L. SAS (SAS Institute, Cary, NC, USA), version 9.4, was used for analyses.

Between group comparisons for Phase 1, Phase 2, and Phase 3 dietary intake and exercise-induced energy expenditure data were assessed using two sample Student's *t*-tests. Baseline and day 14 body weights were compared using a paired t-test to determine whether weight was maintained during Phase 1. Primary analysis of body composition, clinical, muscular function, and muscle fibre cross-sectional area, nuclear peroxisomal proliferator–activated receptor γ coactivator 1α, and aerobic adaptation parameters were performed using a mixed effect linear model. Models include the final measurement day of each phase as the outcome. Treatment (Testosterone and Placebo), phase (Phases 1–3), phase-by-treatment interaction, age, and pre-study values (only for body composition and clinical parameters, Table 1) were considered fixed effects covariates in the model. The random effect included an unstructured covariance matrix to account for the correlation within-subjects over time. Least squares means from the model estimated interaction effects. For outcomes with significant phase-by-treatment interactions, within group comparisons across phases (n = 6) and between group comparisons within each phase (n = 3) were made using two sample Student's *t*-tests based on the least squares means and adjusted using the Bonferroni correction (maximum of 9 comparisons). Unadjusted data are reported in Supplementary Table 4.

3. Results

3.1. Inclusion

Non-obese, young men were enrolled (n = 53), randomised (n = 50), and completed (n = 50) this double-blinded, placebo-controlled trial (Fig. 1, Table 1). Throughout the study, adverse events (Table 2) were not different between Testosterone and Placebo.

3.2. Characteristics and effects during phase 1

During Phase 1, exercise-induced energy expenditure (mean ± standard deviation, Testosterone, 331 ± 387 kcal/d and Placebo, 360 ± 345 kcal/d) and dietary intake did not differ between groups (P > .05, Supplementary Table 1). Participants in both groups remained weight stable [Testosterone, d 0: 81 kg (77, 85) and d 14: 80 kg (76, 85), P = .38; Placebo, d 0: 77 kg (72, 81) and d 14: 76 kg (72, 81), P = .62].

3.3. Characteristics of phase 2

During Phase 2, total daily energy expenditure, exercise-induced energy expenditure, percent and absolute energy deficit, and modality-specific exercise did not differ (P > .05) between groups (Supplementary Tables 2–3). Except for relative protein intake, energy and macronutrient intake did not differ between treatments (Supplementary Table 1).

Table 1
Pre-study participant characteristics in the intention-to-treat population.

	Testosterone (n = 24)	Placebo (n = 26)
Race and ethnicity, n (%)		
Non-Hispanic Black	6 (25)	7 (27)
Non-Hispanic White	14 (58)	16 (61)
Hispanic	3 (13)	0 (0)
Other	1 (4)	3 (12)
Age, y	25 (5)	25 (5)
Height, cm	178 (9)	178 (6)
Weight, kg	81 (14)	77 (10)
Body mass index, kg/m^2	25 (3)	24 (3)
Systolic blood pressure, mmHg	119 (8)	117 (10)
Diastolic blood pressure, mmHg	73 (8)	74 (6)
Total testosterone, nmol/L	17 (5)	15 (3)†
Free testosterone, nmol/L	0.34 (0.10)	0.31 (0.10)
Follicle stimulating hormone, IU/L	3.3 (1.9)	3.8 (2.2)
Estradiol, pmol/L	157 (52)	155 (53)
Sex-hormone binding globulin, nmol/L	37 (18)	32 (11)
Luteinising hormone, IU/L	3.7 (1.3)	4.4 (2.0)
Insulin-like growth factor-1, nmol/L	37 (9)	36 (12)
Glucose, mmol/L	4.9 (0.4)	4.9 (0.3)
Insulin, pmol/L	68 (106)	55 (51)
Cortisol, nmol/L	371 (107)	411 (141)
Total cholesterol, mmol/L	4.2 (0.7)	4.3 (0.9)
High-density lipoprotein cholesterol, mmol/L	1.5 (0.3)	1.4 (0.3)
Low-density lipoprotein cholesterol, mmol/L	2.4 (0.6)	2.6 (0.7)
Triglycerides, mmol/L	0.81 (0.41)	0.87 (0.50)
Haemoglobin, g/L	150 (10)	150 (11)
Haematocrit, %	45 (2)	44 (3)
Prostate-specific antigen, μg/L	0.78 (0.61)	0.67 (0.34)
Alanine aminotransferase, μkat/L	0.39 (0.14)	0.35 (0.13)
Body mass composition, kg		
Total	81.0 (13.7)	76.5 (10.3)
Lean	60.7 (10.1)	56.0 (5.4)*
Fat	17.0 (6.8)	17.4 (6.6)
Peak oxygen uptake, L/min	3.6 (0.8)	3.4 (0.6)
Absolute intake, kcal/d or g/d		
Energy	2432 (777)	2478 (950)
Carbohydrate	258 (99)	281 (114)
Protein	118 (39)	113 (40)
Fat	106 (38)	103 (49)
Relative intake, kcal/kg body mass/d or g/kg body mass/d		
Energy	32 (14)	33 (13)
Carbohydrate	3.4 (1.7)	3.8 (1.6)
Protein	1.5 (0.6)	1.5 (0.6)
Fat	1.4 (0.6)	1.4 (0.7)

Values are mean (standard deviation). Testosterone = 55% energy deficit + 200 mg testosterone enanthate per week during Phase 2, Placebo = 55% energy deficit + 1 mL sesame seed oil placebo per week during Phase 2.

Table 2
Incidence of adverse events in the intention-to-treat population.

	Testosterone (n = 24)			Placebo (n = 26)		
	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3
Death	0	0	0	0	0	0
Serious adverse event						
Low absolute neutrophil count	0	0	0	1	0	0
Elevated alanine aminotransferase	0	0	3	0	0	0
Biopsy infection, cellulitis	0	1	0	0	0	0
Adverse event-related withdrawals	0	0	0	0	0	0
Adverse event						
Biopsy pain	20	20	0	17	23	2
Blister/chafing	0	8	1	0	7	1
Foot pain	0	7	0	0	7	0
Joint/muscle soreness	3	28	0	2	26	0
Gastrointestinal	1	5	1	0	5	1
Dermatological	1	5	1	3	10	0
Insomnia	0	9	0	0	7	0
Headache	2	4	3	0	2	3
Allergy/eye irritation/ear pain	3	2	3	4	5	0
Low haemoglobin, haematocrit, and mean cell volume	0	1	0	0	0	0
Iron deficiency anaemia	0	0	0	0	1	0
Other[1]	1	4	0	2	2	1

Chi square tests were used to compare adverse event incidence in Testosterone (n = 24) and Placebo (n = 26) within each phase. There were no between group differences during any phase (P > .05). Percutaneous muscle biopsies of the vastus lateralis were obtained during P1 and P2. P1 = phase 1, P2 = phase 2, P3 = phase 3, Testosterone = 55% energy deficit + 200 mg testosterone enanthate per week during Phase 2, Placebo = 55% energy deficit + 1 mL sesame seed oil placebo per week during Phase 2. [1]Other: Testosterone; P1, vasovagal/syncope (n = 1); P2, vasovagal/syncope (n = 1), fatigue (n = 1), blurred vision (n = 1), herpes simplex (n = 1); Placebo; P1, chest pain (n = 1), vertigo (n = 1); P2, vertigo (n = 1), dysuria (n = 1); P3, visual disturbance (n = 1).

3.4. Effects of testosterone supplementation on circulating biomarkers during phase 2

Following energy deficit in Phase 2, concentrations of free testosterone decreased, total testosterone remained unchanged, and sex-hormone binding globulin increased in Placebo; whereas, total testosterone and free testosterone increased and sex-hormone binding globulin was unchanged in Testosterone, such that, total testosterone [all bracketed data are mean difference between groups (95% CI), 24.4 nmol/L (21.8, 27.0), P < .0001] and free testosterone [0.69 nmol/L (0.61, 0.77), P < .0001] were greater and sex-hormone binding globulin concentrations [15.9 (11.4, 20.4), P < .0001] were lower in Testosterone than Placebo (Table 3). Estradiol [116 pmol/L (91, 140), P < .0001], follicle stimulating hormone [2.6 IU/L (2.0, 3.2), P < .0001], and luteinising hormone [2.5 IU/L 1.7, 3.4, P < .0001] concentrations were different for Testosterone compared to Placebo (Table 3). Furthermore, Testosterone had higher haemoglobin [6.7 g/L (3.0, 10.3), P < .0048] and haematocrit [2.1% (0.9, 3.2), P < .0053] than Placebo (Table 3).

3.5. Effects of testosterone supplementation on body composition during phase 2

During Phase 2, Placebo lost more body weight than Testosterone (P < .0001, Fig. 3A). Fat mass decreased similarly in both treatment groups (Fig. 3B, Table 3). Bone mineral content did not differ between treatment groups (Table 3). Lean body mass increased in Testosterone, whereas lean body mass remained stable in Placebo, such that lean body mass significantly differed between groups at the end of Phase 2 [2.5 kg (3.3, 1.6); P < .0001]. The change in total testosterone was positively associated with change in lean body mass (r = 0.71, r^2 = 0.50, P < .0001; Supplementary Fig. 1). Similarly, free testosterone was associated with the change in lean body mass (r = 0.69, r^2 = 0.48, P < .001).

3.6. Effects of testosterone supplementation on muscle function, myofibre cross-sectional area, and aerobic adaptations during phase 2

Lower-body muscle function declined similarly for both groups during Phase 2, despite no loss of lean body mass in either group (Table 3). Type I slow-twitch myofibre cross-sectional area and percent myofibre distribution increased, whereas type II fast-twitch myofibre cross-sectional area and percent distribution decreased during Phase 2 as compared to Phase 1, independent of treatment (Fig. 4A–H, all P < .05). Independent of treatment, submaximal aerobic exercise economy was markedly improved for a given matched workload (i.e., greater reliance on fat oxidation and lower perceived effort, heart rate, energy expenditure, and oxygen uptake, all P < .05, Supplementary Table 5). There were no effects of energy deficit or treatment on basal (fasted, rested) nuclear percentage of peroxisomal proliferator–activated receptor γ coactivator 1α [Testosterone, d 14: 42.6% (33.4, 51.8) and d 42: 48.2% (38.1, 58.2); Placebo, d 14: 50.7% (41.9, 59.6) and d 42: 52.4% (42.8, 62.1), P-interaction = 0.42].

Table 3
Adjusted least squares means for body composition, clinical biomarkers, and lower-body muscular strength and endurance in the intention-to-treat population.

	Testosterone (n = 24)			Placebo (n = 26)			P-value		
	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3	Phase	Treat	Phase × Treat
Body composition									
Body mass, kg									
Total	78.0 (77.4, 78.7)[a]	75.8 (75.1, 76.5)[b,*]	79.3 (78.6, 79.9)[a,*]	78.3 (77.7, 78.9)[a]	73.3 (72.7, 74.0)[b]	76.5 (75.8, 77.1)[c]	<0.0001	<0.0001	<0.0001
Lean	57.9 (57.3, 58.5)[a]	60.4 (59.8, 61.0)[b,*]	63.1 (62.5, 63.7)[c,*]	58.3 (57.7, 58.9)[a]	58.0 (57.4, 58.6)[a]	60.5 (60.0, 61.1)[b]	<0.0001	<0.0001	<0.0001
Fat[1]	16.8 (16.3, 17.2)	12.0 (11.6, 12.4)	12.8 (12.4, 13.2)	16.8 (16.4, 17.2)	12.2 (11.8, 12.6)	12.8 (12.4, 13.2)	<0.0001	0.78	0.79
Bone mineral content, kg	3.27 (3.26, 3.28)[a]	3.27 (3.26, 3.28)[a]	3.26 (3.25,3.27)[a]	3.27 (3.26, 3.28)[a]	3.25 (3.24, 3.27)[a]	3.24 (3.23, 3.25)[b,*]	<0.0001	0.016	0.035
Leg mass, kg									
Total	26.7 (26.4, 27.0)[a]	25.8 (25.5, 26.1)[b]	26.9 (26.6, 27.2)[a,*]	26.8 (26.5, 27.1)[a]	25.2 (24.9, 25.5)[b]	26.1 (25.8, 26.4)[c]	<0.0001	0.0089	0.012
Lean	19.5 (19.3, 19.8)[a]	20.1 (19.8, 20.4)[b,*]	21.2 (20.9, 21.4)[c,*]	19.6 (19.3, 19.8)[a]	19.3 (19.0, 19.6)[a]	20.2 (19.9, 20.4)[b]	<0.0001	0.0001	<0.0001
Fat	6.0 (5.8, 6.1)[a]	4.4 (4.3, 4.6)[b]	4.5 (4.4, 4.6)[b]	6.0 (5.8, 6.1)[a]	4.7 (4.6, 4.8)[b]	4.7 (4.6, 4.9)[b]	<0.0001	0.039	0.015
Trunk mass, kg									
Total	35.7 (35.3, 36.1)[a]	34.8 (34.3, 35.2)[b,*]	36.7 (36.3, 37.1)[c*]	35.9 (35.5, 36.2)[a]	33.4 (33.0, 33.8)[b]	35.1 (34.7, 35.5)[a]	<0.0001	<0.0001	<0.0001
Lean	26.7 (26.3, 27.1)[a]	28.5 (28.1, 28.8)[b,*]	29.8 (29.5, 30.2)[c,*]	27.0 (26.6, 27.3)[a]	27.4 (27.1, 27.8)[a]	28.7 (28.4, 29.1)[b]	<0.0001	0.0018	<0.0001
Fat[1]	8.0 (7.7, 8.3)	5.3 (5.0, 5.6)	5.9 (5.6, 6.2)	8.0 (7.7, 8.3)	5.1 (4.8, 5.4)	5.6 (5.3, 5.8)	<0.0001	0.28	0.39
Clinical health-biomarkers									
Total testosterone, nmol/L	15.5 (13.7, 17.4)[a]	36.2 (34.3, 38.0)[b,*]	7.8 (5.9, 9.8)[c,*]	14.6 (12.9, 16.4)[a]	11.8 (10.0, 13.5)[a]	14.6 (12.8, 16.3)[a]	<0.0001	<0.0001	<0.0001
Free testosterone, nmol/L	0.33 (0.27, 0.38)[a]	0.87 (0.81, 0.92)[b,*]	0.15 (0.10, 0.21)[c,*]	0.31 (0.26, 0.36)[a]	0.18 (0.13, 0.23)[b]	0.29 (0.23, 0.34)[a,b]	<0.0001	<0.0001	<0.0001
Follicle stimulating hormone, IU/L	3.1 (2.7, 3.6)[a]	0.3 (−0.2, 0.7)[b,*]	2.2 (1.7, 2.7)[c,*]	3.5 (3.0, 3.9)[a]	2.8 (2.4, 3.3)[a]	3.5 (3.0, 3.9)[a]	<0.0001	<0.0001	<0.0001
Estradiol, pmol/L	148 (130, 166)[a]	247 (229, 265)[b,*]	144 (125, 162)[a]	142 (124, 159)[a]	131 (114, 148)[a]	158 (141, 175)[a]	<0.0001	<0.0001	<0.0001
Sex-hormone binding globulin, nmol/L	31.6 (28.3, 34.8)[a,b]	36.5 (33.2, 39.7)[a,*]	30.6 (27.2, 34.0)[b]	31.1 (27.9, 34.2)[a]	52.4 (49.2, 55.5)[b]	35.7 (32.6, 38.8)[a]	<0.0001	0.0001	<0.0001
Luteinizing hormone, IU/L	3.3 (2.6, 3.9)[a]	0.3 (−0.4, 0.9)[b,*]	2.2 (1.6, 2.9)[a,*]	3.8 (3.2, 4.3)[a,b]	2.8 (2.2, 3.4)[a]	4.1 (3.5, 4.7)[b]	<0.0001	<0.0001	0.0006
Insulin-like growth factor-1, nmol/L[2]	38.5 (35.6, 41.5)	25.0 (22.0, 27.9)	36.6 (33.5, 39.7)	38.2 (35.3, 41.0)	25.8 (22.9, 28.6)	37.5 (34.7, 40.4)	<0.0001	0.73	0.89
Glucose, mmol/L[1]	4.71 (4.53, 4.88)	4.14 (3.96, 4.32)	4.93 (4.74, 5.11)	4.63 (4.46, 4.81)	4.19 (4.02, 4.36)	4.91 (4.74, 5.08)	<0.0001	0.88	0.76
Insulin, pmol/L[2]	59 (35, 83)	15 (−9, 39)	80 (55, 105)	54 (31, 77)	11 (−12, 34)	87 (64, 110)	<0.0001	0.93	0.86
Cortisol, nmol/L[2]	397 (356, 438)	512 (472, 553)	388 (345, 430)	434 (395, 473)	551 (512, 590)	378 (339, 417)	<0.0001	0.73	0.89
Total cholesterol, mmol/L[1]	3.85 (3.59, 4.11)	4.39 (4.13, 4.65)	4.97 (4.70, 5.24)	3.86 (3.61, 4.11)	4.12 (3.87, 4.37)	4.98 (4.73, 5.23)	<0.0001	0.54	0.22
High-density lipoprotein cholesterol, mmol/L	1.22 (1.11, 1.32)[a]	1.64 (1.54, 1.75)[b]	1.85 (1.74, 1.96)[c]	1.22 (1.12, 1.32)[a]	1.75 (1.65, 1.85)[b]	1.74 (1.64, 1.84)[b]	<0.0001	0.98	0.008
Low-density lipoprotein cholesterol, mmol/L	2.23 (2.03, 2.43)	2.44 (2.24, 2.63)	2.74 (2.53, 2.95)	2.29 (2.10, 2.48)	2.14 (1.95, 2.34)	2.78 (2.59, 2.98)	<0.0001	0.53	0.054
Triglyceride, mmol/L	0.84 (0.69, 0.99)[a]	0.65 (0.49, 0.80)[a]	0.78 (0.62, 0.94)[a]	0.80 (0.66, 0.95)[a,b]	0.52 (0.37, 0.67)[a]	1.03 (0.88, 1.17)[b]	0.0001	0.63	0.038
Haemoglobin, g/L	145 (143, 148) [a]	145 (142, 148)[a,*]	145 (142, 147)[a]	144 (142, 147)[a]	138 (136, 141)[b]	139 (137, 142)[b]	0.012	0.002	0.035
Haematocrit, %	42.9 (42.1, 43.7)[a]	43.6 (42.7, 44.4)[a,*]	43.2 (42.3, 44.0)[a]	42.8 (42.0, 43.6)[a]	41.5 (40.7, 42.3)[a]	41.5 (40.7, 42.3)[a]	0.45	0.003	0.020
Alanine aminotransferase, μkat/L[3]	0.32 (−0.20, 0.83)	0.42 (−0.09, 0.94)	2.65 (2.11, 3.18)	0.38 (−0.12, 0.87)	0.49 (0.00, 0.98)	1.68 (1.19, 2.18)	<0.0001	0.19	0.074
Prostate-specific antigen, μg/L[1]	0.77 (0.56, 0.98)	0.68 (0.47, 0.89)	–	0.59 (0.39, 0.79)	0.43 (0.23, 0.63)	–	<0.0001	0.14	0.15
Systolic blood pressure, mm Hg[1]	104 (99, 108)	99 (94, 103)	–	103 (98, 107)	98 (94, 102)	–	0.0002	0.80	0.75
Diastolic blood pressure, mm Hg[1]	63 (60, 66)	58 (55, 60)	–	64 (61, 67)	58 (55, 60)	–	<0.0001	0.73	0.66
Lower-body muscular strength and endurance									
Isometric torque									
Peak, Nm[4]	245 (221, 269)	219 (195, 243)	214 (190, 238)	243 (220, 266)	219 (196, 243)	229 (205, 252)	<0.0001	0.78	0.37
Peak, Nm/kg lean body mass[4]	24 (22, 26)	21 (19, 23)	20 (18, 22)	26 (24, 28)	24 (22, 26)	24 (22, 26)	<0.0001	0.014	0.31
Isokinetic torque									
Peak, Nm[4]	200 (181, 219)	164 (145, 183)	174 (155, 193)	195 (177, 213)	163 (145, 181)	168 (150, 186)	<0.0001	0.72	0.82
Peak, Nm/kg lean body mass[4]	20 (18, 21)	16 (14, 17)	16 (14, 17)	21 (19, 22)	17 (16, 19)	17 (16, 19)	<0.0001	0.12	0.72
Total work, J[1]	3339 (3024, 3653)	2800 (2485, 3115)	3180 (2865, 3494)	3423 (3121, 3726)	2843 (2541, 3146)	2985 (2683, 3288)	<0.0001	0.91	0.26
Total work, J/kg lean body mass[4]	327 (303, 352)	266 (242, 290)	291 (267, 316)	362 (339, 385)	306 (282, 329)	307 (284, 331)	<0.0001	0.033	0.33

Values are least squares mean (95% confidence interval). Data were analysed using linear mixed models, adjusted for age (all parameters) and pre-study value (only for body composition and clinical parameters). Bonferroni corrections were used for post hoc comparisons. Data not sharing the same letter superscript within a treatment are different by phase and *indicates a between group difference at a particular phase (phase-by-treat interaction). Main effect of phase, [1]all phases are different, [2]Phase 1 and Phase 3 are different than Phase 2, [3]Phase 1 and Phase 2 are different than Phase 3, [4]Phase 1 is different than Phase 2 and Phase 3. $P < .05$ considered statistically significant. Testosterone = 55% energy deficit + 200 mg testosterone enanthate per week during Phase 2, Placebo = 55% energy deficit + 1 mL sesame seed oil placebo per week during Phase 2.

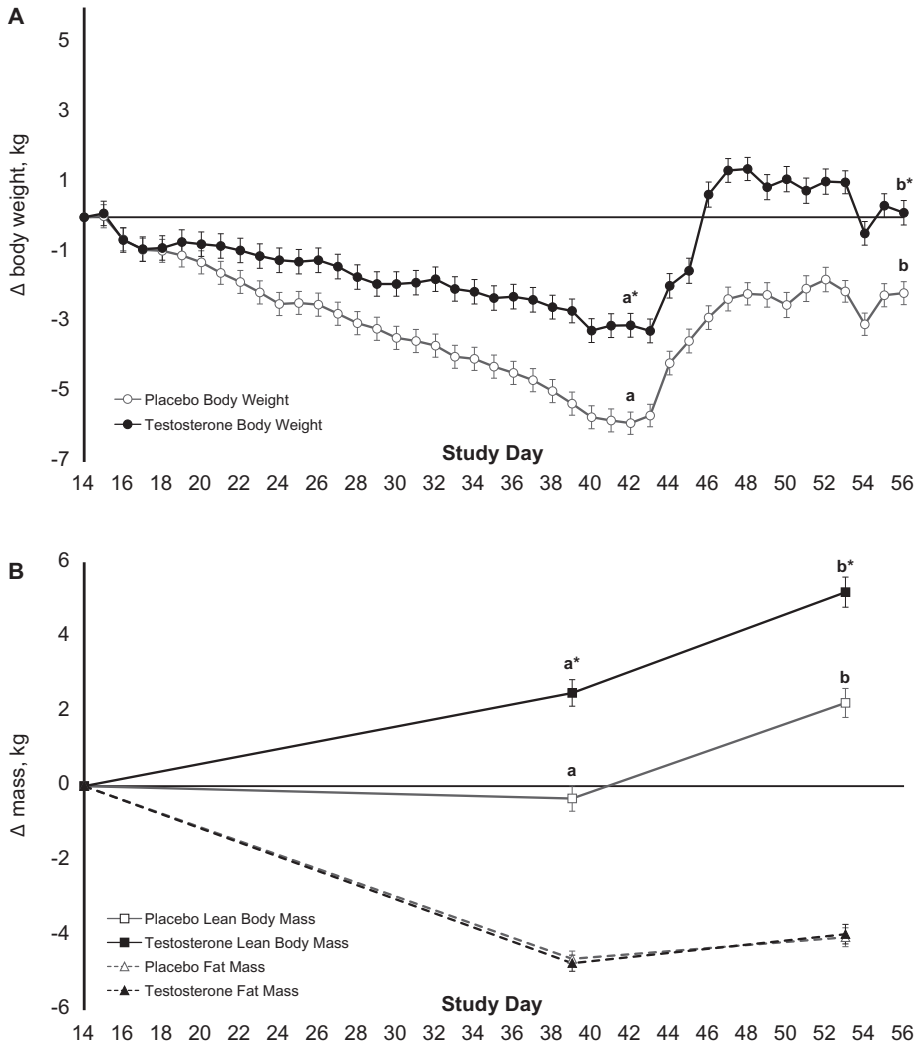

Fig. 3. Change in body weight and composition during energy deficit and recovery. Least squares mean ± standard error change in body weight (A) and composition (B) during Phase 2 (55% energy deficit) and Phase 3 (recovery/weight regain) relative to the final body weight and composition measured during Phase 1 (eucaloric diet) for Testosterone (n = 24), 55% energy deficit + 200 mg testosterone enanthate per week during Phase 2 (n = 24) and Placebo, 55% energy deficit + 1 mL sesame seed oil placebo per week during Phase 2 (n = 26). Data were analysed using linear mixed models, adjusting for age and pre-study value. Bonferroni corrections were used for post hoc comparisons. Data not sharing the same letter superscript within a treatment are different; and *indicates a between group difference (phase-by-treatment interaction).

3.7. Characteristics and effects during phase 3

During Phase 3, ad-libitum dietary intake was not different between groups (Supplementary Table 1). Total testosterone [6.7 nmol/L (4.1, 9.4), P < .0001], free testosterone [0.13 nmol/L (0.05, 0.21), P < .013], and follicle stimulating hormone [1.3 IU/L (0.6, 1.9), P < .0011] concentrations were lower for Testosterone than Placebo (Table 3), but no other health-related biomarkers differed by group. Both groups regained body weight; however, body weight remained greater in Testosterone than Placebo (P < .0001; Fig. 3A). Both groups also gained lean body mass, but lean body mass remained greater in Testosterone than Placebo [2.6 kg (1.8, 3.4), P < .0001; Fig. 3B, Table 3]. Bone mineral content decreased in Placebo and remained stable in Testosterone, such that bone mineral content significantly differed between groups at the end of Phase 3 [21 g [7,36]; P < .043]. Fasting insulin concentrations were greater following recovery compared to the energy deficit, with no difference between groups. Muscle function measures remained lower during Phase 3 than Phase 1, regardless of treatment allocation (P < .0001).

4. Discussion

In the current proof-of-concept study, we report that during 28-d of severe exercise- and diet-induced energy deficit, non-obese, young-adult males receiving weekly intramuscular injections of testosterone (200 mg testosterone enanthate/wk) gained lean body mass and lost less total mass compared to those receiving placebo injections. Testosterone fully recovered their body weight and were 2.8 kg heavier at the end of the 14-d recovery period than Placebo, due almost entirely to lean body mass. Testosterone did not differ from Placebo with respect to lower-body muscular strength and endurance, incidence of adverse events, systolic or diastolic blood pressure, or glucose, insulin, cortisol, lipid, lipoprotein, insulin-like growth factor 1, alanine aminotransferase, or prostate-specific antigen concentrations during energy deficit or recovery.

Suppression of the hypothalamic-pituitary-gonadal axis in energy deficient, non-obese men is well-documented, particularly during sustained, strenuous military operations [1,23,24]. However, no study, to our knowledge, has attempted to replicate these testosterone

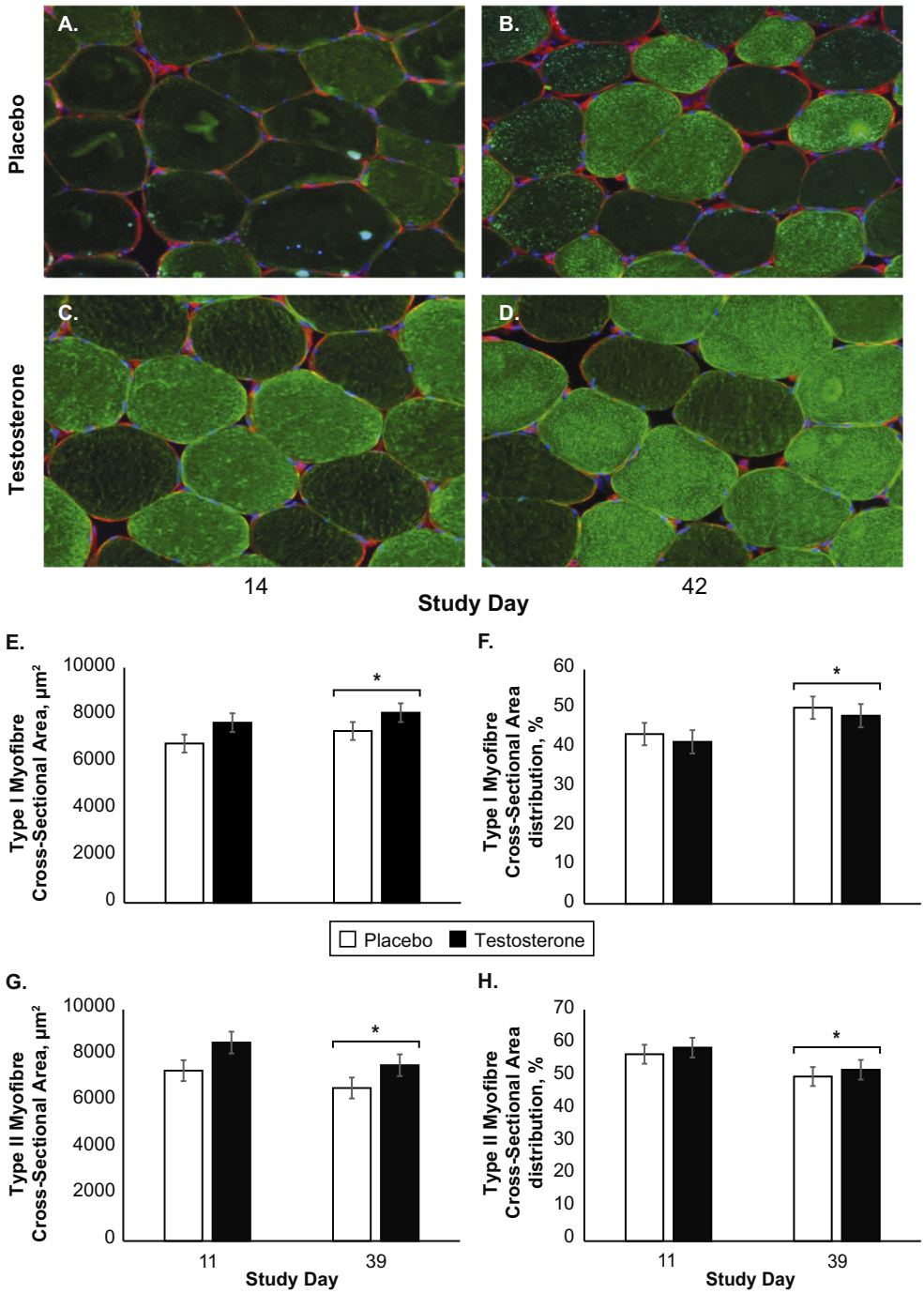

Fig. 4. Muscle fibre type and percent distribution during energy balance and energy deficit. Representative images of serial cross-sections from vastus lateralis samples collected at rest, under fasted conditions during phase 1 (eucaloric phase, day 14) and phase 2 (55% energy deficit, day 42) for Placebo (A–B), 55% energy deficit + 1 mL sesame seed oil placebo per week during Phase 2 (n = 26) and Testosterone (C–D), 55% energy deficit + 200 mg testosterone enanthate per week during Phase 2 (n = 24). Images were taken with the 20× objective lens in a 15 × 10 grid. Laminin signals were stitched into one image to cover the entire specimen and analysed using Cell Profiler. Myosin heavy chain myofibres with a median intensity < 0.3 and those ≥0.3 were delineated as type II fast-twitch myofibres and type I slow-twitch myofibres, respectively. Least squares mean ± standard error in type 1 slow-twitch myofibre cross-sectional area (E), type 1 slow-twitch myofibre percent distribution (F), type II fast-twitch myofibre cross-sectional area (G), and type II fast-twitch myofibre percent distribution (H). Data were analysed using linear mixed models, adjusting for age. *Indicates a between phase difference (phase main effect).

decrements in a tightly controlled clinical trial using military-relevant exercise and diet stressors. The inability of our severe exercise- and diet-induced energy deficit to reduce total testosterone concentrations to levels measured during US Army Ranger School [1], the basis for the design of the current study, provides 1) controlled scientific evidence to counter several of the points made in the paper by Friedl and colleagues [1], and 2) valuable information regarding the design of future trials attempting to induce non-pharmacologic decrements in endogenous testosterone status in a clinical setting.

In the current study, the 28-d exercise- and diet-induced energy deficit intervention was modelled to reflect the exercise- and diet-induced stress experienced by US Army Soldiers participating in US Army Ranger School [1], an example of severe military operational stress. Friedl and colleagues [1] suggested that the energy deficit alone, caused by increased energy expenditure and restricted dietary intake, was the primary determinant for the decline in total testosterone. This reasoning was based on the observed restoration of eugonadal status in Soldiers fed adequate energy following the first 28 d of Army Ranger School,

but still exposed to the remaining stressors (i.e., prolonged and strenuous physical work, sleep deprivation, and psychological stress) [1]. The authors concluded that the energy deficit (~1100 kcal/d) alone, not the means by which the deficit was produced, was the primary determinant of the 50–65% decline in total testosterone observed during the first 28-d of US Army Ranger School. Therefore, the current study, which induced a greater daily energy deficit (~1950 kcal), was expected to elicit a similar decline in total testosterone and lean body mass.

This discrepancy between studies may suggest that, in addition to energy restriction and strenuous physical work, other factors, such as sleep deprivation, psychological stress, and the type of physical work employed meaningfully contribute to declines in testosterone status observed during strenuous military training. During real-world military operations, recovery is minimal and military personnel are often sleep deprived. In the current study, psychological stress was not imposed, and sleep was not restricted. Sleep restriction (5 h/night) has been shown to suppress total testosterone by as much as 15% [25]. In a study that involved strenuous military training and produced a substantial energy deficit, continuous sleep (3 h/d), compared to non-continuous sleep, attenuated reductions in total testosterone [24]. Furthermore, discrete aerobic-type exercise bouts were used to increase energy expenditure (3.5 sessions per day, averaging 2.5 h/d) during the 28-d energy deficit, followed by periods of rest. Energy deficits during real-world military operations often result from low-to-moderate increases in metabolic demand and are sustained for long periods, as physical/aerobic exercise-type activity can exceed 10 h/d. As such, the stressors applied in the current study may not have replicated the multi-faceted stress experienced during real-world military operations, which generally produce marked suppression of the hypothalamic-pituitary-gonadal axis and catabolism of lean body mass [6,7,26]. These realizations are critical for the design of future interventional studies of military operational stress and add important evidence to the literature regarding endogenous testosterone decrements that occur during physiologically and psychologically stressful events.

Finally, while the magnitude of the total testosterone decline (19%, NS) in the current study was less than that reported by others [1,27], the significance of a 42% decline in free testosterone – i.e., the bioactive form of testosterone – should be highlighted. Recent guidance from the US Endocrine Society recommends that free testosterone be relied upon for diagnosis of low testosterone in conditions where sex-hormone binding globulin is altered and total testosterone concentrations are borderline low [28,29]. For Placebo, sex-hormone binding globulin increased by 68% during Phase 2 compared to Phase 1 and total testosterone concentrations were borderline low [<13.9 nmol/L, [28]] at the end of Phase 2. Importantly, free testosterone concentrations [0.18 (0.13, 0.23) nmol/L] were below the lower-end of the normal range estimated using the Vermeulen equation [0.25–0.785 nmol/L, [30]] following Phase 2. Therefore, a more holistic examination of the data (i.e., total and free testosterone and sex-hormone binding globulin) suggests testosterone status was degraded by the severe exercise- and diet-induced energy deficit, albeit not to the same degree as during US Army Ranger School.

The hypertrophic effects of supplemental testosterone are well-described [31]. Bhasin et al. [32] reported a 5% (2.5 kg) and 9% (5.5 kg) increase in lean mass in non-obese, young men receiving weekly injections of 125 mg and 300 mg, respectively, of testosterone enanthate while consuming a eucaloric diet for 20-wk. We report a similar increase in lean body mass (~4%, 2.5 kg) after 4-wk in young men receiving 200 mg/wk. of testosterone enanthate while being underfed. The hypertrophic effects of testosterone, despite energy deficit, may be due to enhanced commitment of satellite cells to myogenesis [33] or androgen receptor-mediated cell signalling [34]. Given the magnitude of energy deficit in the current study, preservation of lean body mass in Placebo was unexpected. However, the combination of higher-protein feeding and high-volume exercise may, in part, explain this finding, due to the synergistic restorative effects of dietary protein and mechanical stress on muscle protein synthesis during energy deficit [20,35–37], providing the conditions by which lean body mass may be maintained [38]. The addition of supplemental testosterone may have provided an anabolic stimulus that was additive to the stimulus provided by protein and exercise, explaining, in part, the gain in lean body mass in Testosterone. This hypothesis is supported by studies showing that testosterone administration enhances muscle protein synthetic efficiency, meaning, for a given amount of intracellular essential amino acids, a greater proportion is routed towards protein synthesis than towards oxidative metabolism, thus conserving lean body mass [39,40].

Lower-body muscle function deteriorated similarly between treatments during the 28-d exercise- and diet-induced energy deficit. The decline in muscle function may be attributable to neuromuscular fatigue, secondary to multiple daily submaximal aerobic exercise sessions for 28 d [41]. Reduced motivation over time may also be a factor contributing to the observed decline in muscular strength and endurance [42,43]. However, the incongruence between the lean body mass change and muscle function in our study is not a unique observation. A similar inability to detect differences in muscle function with testosterone supplementation, despite greater lean mass, has also been demonstrated in young men receiving exogenous testosterone during 28 d of bed rest [44], in middle-aged, obese males receiving exogenous testosterone for 56 wk [10], and in older, overweight males receiving testosterone supplementation for 6 mo [45]. However, the lack of a functional benefit should not discount the potential advantage that may be gained by increasing or maintaining lean body mass in response to severe energy deficit. Accrual or preservation of muscle protein mass may afford greater physiological resiliency during severe military operational stress, particularly when repeated bouts of stress are unavoidable, and recovery is limited. For example, stress-mediated lean body mass losses approaching 10% have been associated with impaired immune function [46]. Lean body mass can be viewed as an amino acid reservoir, and increased protein mass, even to a limited extent, may prolong physiological homeostasis during periods of severe energy deficit.

The lack of a quantitative functional benefit for Testosterone despite gains in lean body mass may also raise methodological concerns about the use of DXA for body composition measurements. DXA is the most commonly employed body composition measurement tool in biomedical research [47], yet it provides estimates for only three body compartments (i.e., bone, fat, and lean soft tissue), and does not discern skeletal muscle mass from body water. If gains in lean body mass in Testosterone were due, at least in part, to an increase in extracellular water [48] and muscle glycogen content [49], and not solely to an increase in protein, those changes would not be delineated by DXA. Assessing total body water in combination with DXA (i.e., four compartment model) may have mitigated those concerns. If lean mass hydration is conservatively estimated to be 75%, total body protein accretion in TEST would be ~625 g. Gains in protein mass, without gains in muscle function, may be a reflection of the specific myofibres (i.e., protein fraction) affected by the intervention. More specifically, the reduction in type II fast-twitch myofibre cross-sectional area would support the functional declines observed with the isokinetic and isometric functional measures. The increase in type I slow-twitch myofibre cross-sectional area is consistent with the observed improvement in submaximal aerobic exercise economy. These findings, in addition to the possibility that gains in lean mass were due to fluid or non-contractile protein, may help explain why lean body mass accrual in Testosterone had no functional benefit.

5. Limitations

While the findings from the current study are strengthened by 100% retention and ≥89% adherence with diet and exercise prescriptions during the energy deficit phase, there are limitations to acknowledge, in addition to those already discussed with regards to DXA methodology and muscle function measures. This study was designed to provide

testosterone supplementation at a dose that maintained total and free testosterone concentrations during a severe energy deficit, in order to attenuate expected lean body mass loss. However, following testosterone supplementation in Testosterone, total and free testosterone concentrations were 134% and 164% higher, respectively, than concentrations prior to supplementation. In addition, following Phase 2, total testosterone concentrations in Testosterone were approximately 14% above the upper-end of the harmonized reference range for testosterone (9.2–31.8 nmol/L) recently established by the Endocrine Society in 2018 [28]. Free testosterone concentrations were 11% above the upper-end of the reference range for free testosterone (0.25–0.785 nmol/L) established by Vermeulen and colleagues [18,30]. The increases in total and free testosterone above basal concentrations and the upper-end of the normal reference range were likely due to the administration of a greater dosage than the recommended initial dose for testosterone replacement therapy [28] and, possibly, the discrepancy between actual endogenous testosterone suppression produced by the severe energy deficit and the anticipated suppression, on which the chosen dosage was based [32]. As discussed previously, the anticipated suppression was based on military studies demonstrating marked reductions in total testosterone during demanding military operations that produced severe energy deficits and lean body mass loss [1,27,50]. Instead, we observed lean body mass gains and maintenance in Testosterone and Placebo, respectively; whereas, both groups lost the same amount of body fat. Administering a lower testosterone dose, implementing more demanding stressors that better mimic the multi-stressor environment of real-world military operations, or a combination of both may have facilitated greater changes in testosterone status and lean body mass.

The dose and duration of exogenous supplementation dictates the recovery of endogenous testosterone production [28]. In the current study, total testosterone concentrations in Testosterone were ~78% and ~50% lower following recovery compared to the energy deficit and run-in phases, respectively. Similarly, free testosterone concentrations in Testosterone were ~83% and ~55% lower following Phase 3 compared to Phases 1 and 2, respectively. Despite lower circulating total and free testosterone concentrations in Testosterone compared to Placebo, lean body mass remained greater in Testosterone than Placebo. However, muscle function measures remained lower in recovery than during the run-in phase and similar to the measures taken at the end of Phase 2, regardless of treatment group. Total and free testosterone concentrations were; however, restored to normal levels within 28 d ($n = 13$), 90 d ($n = 8$), and 11 mo ($n = 1$) after recovery (2 participants did not return for follow-up) [14]. The potential physiological and psychological consequences of supplemental testosterone-induced hypogonadism and subsequent endogenous and functional recovery require consideration. Finally, the benefits and risks of using supplemental testosterone to aid in total body mass and lean body mass retention and muscle function during and in recovery from severe energy deficit in non-obese, young-adult males beyond the 4 wk energy deficit and 2 wk recovery period studied in this trial are unknown.

6. Conclusions

Exogenous testosterone, to our knowledge, has not been administered as an intervention to counter anticipated reductions in testosterone status, lean body mass, and muscle function due to military operational stress. Findings from this proof-of-concept study show testosterone supplementation increases total and free testosterone, and lean body mass but has no effect on lower-body muscle function, type I slow-twitch and type II fast-twitch myofibre cross-sectional area, whole-body and muscle aerobic adaptations, circulating health biomarkers, or blood pressure during a tightly controlled clinical study resembling military operational stress that is severe, but less so than US Army Ranger School. This indicates testosterone supplementation is likely not warranted for operational scenarios that do not markedly decrease testosterone status and, when designing future studies, more sensitive outcomes to assess muscle function and physical performance (e.g., anaerobic/aerobic capacity, occupational and tactical military tasks, including timed load carriage marches, casualty drag, litter carries, and marksmanship) should be included. The primary findings from this study provide rationale for further exploration of practical pharmacologic interventions to mitigate losses of lean body mass experienced by military personnel during periods of unavoidable, severe energy deficit, particularly those that may enhance muscle function.

Contributors

Conceptualization, S.M.P., C.E.B., J.P.K., H.R.L., J.S.O., L.M.M., J.A.C., A.J.Y., M.A.M., W.J.E., O.V., O.T.C., K.M.G., and J.C.R.; Formal Analysis, S.M.P., C.E.B., R.A.B., and J.C.R.; Investigation, S.M.P., C.E.B., N.M.J., M.N.H., and J.C.R.; Writing – Original Draft, S.M.P., C.E.B., and J.C.R.; Writing – Reviewing & Editing, J.P.K., H.R.L., J.S.O., L.M.M., J.A.C., A.J.Y., M.A.M., W.J.E., O.V., O.T.C., K.M.G., N.M.J., R.A.B., and M.N.H.; Supervision,; Project Administration, S.M.P., C.E.B., K.M.G., M.N.H., and J.C.R.; Funding Acquisition, S.M.P. and J.C.R.

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Declaration of Competing Interest

J.C.W., O.T.C., and K.M.G. reported that their institution received funding from the US Department of Defence for work associated with this publication. H.R.L. reported receiving personal fees from Pfizer, Inc., for work outside this publication. All remaining authors declare no competing interests. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or the Department of Defence. Any citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement of approval of the products or services of these organizations.

Acknowledgements

The research reported in this publication is supported by the Collaborative Research to Optimize Warfighter Nutrition II and III projects and the Joint Program Committee-5, funded by the US Department of Defence. The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication. The full trial protocol can be accessed as online Supplementary material. The authors wish to thank the participants who consented and participated in this research experiment. The authors would like to thank Dr. Shalender Bhasin for sharing his expertise and assisting the research team on the design of the testosterone intervention. We would also like to thank the study team at Pennington Biomedical Research Centre for their significant contributions to study management and conduct, data collection, and analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2019.07.059.

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Testosterone Administration During Energy Deficit Suppresses Hepcidin and Increases Iron Availability for Erythropoiesis

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Context: Severe energy deprivation markedly inhibits erythropoiesis by restricting iron availability for hemoglobin synthesis.

Objective: The objective of this study was to determine whether testosterone supplementation during energy deficit increased indicators of iron turnover and attenuated the decline in erythropoiesis compared to placebo.

Design: This was a 3-phase, randomized, double-blind, placebo-controlled trial.

Setting: The study was conducted at the Pennington Biomedical Research Center.

Patients or Other Participants: Fifty healthy young males.

Intervention(s): Phase 1 was a 14-day free-living eucaloric controlled-feeding phase; phase 2 was a 28-day inpatient phase where participants were randomized to 200 mg testosterone enanthate/week or an isovolumetric placebo/week during an energy deficit of 55% of total daily energy expenditure; phase 3 was a 14-day free-living, ad libitum recovery period.

Main Outcome Measure(s): Indices of erythropoiesis, iron status, and hepcidin and erythroferrone were determined.

Results: Hepcidin declined by 41%, indicators of iron turnover increased, and functional iron stores were reduced with testosterone administration during energy deficit compared to placebo. Testosterone administration during energy deficit increased circulating concentrations of erythropoietin and maintained erythropoiesis, as indicated by an attenuation in the decline in hemoglobin and hematocrit with placebo. Erythroferrone did not differ between groups, suggesting that the reduction in hepcidin with testosterone occurs through an erythroferrone-independent mechanism.

Conclusion: These findings indicate that testosterone suppresses hepcidin, through either direct or indirect mechanisms, to increase iron turnover and maintain erythropoiesis during severe energy deficit. This trial was registered at www.clinicaltrials.gov as #NCT02734238. **(*J Clin Endocrinol Metab* 105: e1316–e1321, 2020)**

Key Words: testosterone, erythropoiesis, hepcidin, iron

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA
Published by Oxford University Press on behalf of the Endocrine Society 2020. This work is written by (a) US Government employee(s) and is in the public domain in the US.
Received 19 October 2019. Accepted 30 December 2019.
First Published Online 2 January 2020.
Corrected and Typeset 11 March 2020.

Steady-state erythropoiesis in healthy human adults produces ~200 billion new erythrocytes each day, a process that requires 20 mg to 25 mg of iron daily (1). Erythropoiesis is inhibited during severe energy

deprivation (2–5), which can occur during military training and operations (6). Diminished erythropoiesis as a result of energy deficit is accompanied by decreases in erythroid progenitors in the bone marrow to preserve iron stores for vital functions. This response underlies the pathogenesis of iron-restricted anemias, such as iron deficiency anemia and the anemia of chronic disease and inflammation (7).

Testosterone is a potent stimulator of erythropoiesis (8–12). In fact, prior to the advent of recombinant erythropoietin (EPO), androgens were used to treat iron deficiency anemia and the anemia of chronic disease and inflammation (8). However, the impact and mechanisms by which testosterone affects erythropoiesis in humans during severe energy deficit remains to be determined. Energy-restricted mice injected with testosterone have increased ^{59}Fe incorporation into newly formed erythrocytes, an indirect measure of erythropoiesis, compared to energy restriction alone (13). Although this suggests that testosterone administration may be an effective strategy to maintain erythropoiesis during energy deficit in mice, to our knowledge, this has not been tested in humans. The objective of this study was to determine the effects of testosterone supplementation, compared to placebo, on erythropoiesis and iron homeostasis in healthy young males during energy deficit.

Materials and Methods

This study addresses secondary objectives of the Optimizing Performance for Soldiers study (14, 15). The study conformed to the principles in the Declaration of Helsinki and was approved by the Institutional Review Board of the Pennington Biomedical Research Center and the U.S. Army Human Research Protections Office. Participants provided written and voluntary informed consent (ClinicalTrails.gov #NCT02734238).

Participants

Healthy, physically active males with normal total testosterone concentrations (10.4–34.7 nmol/L) were recruited. Additional inclusion/exclusion criteria are reported elsewhere (14). Fifty-three participants were enrolled, 50 randomized, and 50 completed the study (15).

Study design and procedures

This study was a 3-phase, randomized, double-blind, placebo-controlled trial. Prestudy characteristics of participants are shown in Table 1. Phase 1 was a 2-week (days 1–14), free-living, controlled-feeding phase. Phase 1 diets were designed to maintain body mass and provide 1.6 g protein/kg/day and 30% fat. Average iron content of the diets during phase 1 were (mean ± SD) 18.8 ± 7.9 mg/day and 19.6 ± 13.4 mg/day for the placebo and testosterone groups, respectively (P = 0.81).

Table 1. Prestudy characteristics of study participants

	Placebo (n = 26)	Testosterone (n = 24)
Race and ethnicity, n (%)		
Non-Hispanic Black	7 (27)	6 (25)
Non-Hispanic White	16 (61)	14 (58)
Hispanic	0 (0)	3 (13)
Other	3 (12)	1 (4)
Age, y	25 ± 1	25 ± 1
Weight, kg	76 ± 2	81 ± 3
BMI, kg/m^2	24 ± 1	25 ± 1
Fat mass, %	17.4 ± 1.3	17.0 ± 1.4
Lean mass, %	56.0 ± 1.1	60.6 ± 2.1*
VO_{2peak}, L/min	3.4 ± 0.6	3.6 ± 0.8
Dietary iron, mg/d	18.8 ± 7.9	19.1 ± 13.3
Energy, kcal/d	2478 ± 950	2432 ± 777

Differences at baseline (day 0) were analyzed using Student's t-tests. Values are means ± SD or n (%). *P < 0.05 compared to placebo. Abbreviation: BMI, body mass index; VO_2, volume of oxygen uptake.

Phase 2 was a 4-week (days 15–42), inpatient, controlled exercise and feeding phase. At the start of phase 2, participants were randomized to receive either intramuscular injection of testosterone enanthate (200 mg/week) or placebo (1 mL sesame oil/week) on days 15, 21, 28, and 35. Participants were provided a diet and exercise prescription that resulted in a 55% energy deficit during phase 2. Dietary energy intake was restricted to 45% and total daily energy expenditure was increased by 50% of phase 1 total daily energy expenditure. The macronutrient composition of the phase 2 diet was consistent with phase 1 and did not differ between groups. Average iron content of the diets during phase 2 were 17.1 ± 3.5 mg/day and 17.8 ± 4.0 mg/day for the placebo and testosterone groups, respectively (P = 0.52). To increase phase 2 total daily energy expenditure, participants performed low-, moderate-, and high-intensity (40–85% of predetermined peak rate of oxygen uptake) aerobic-type exercise (eg, treadmill and/or outdoor walking and/or running, elliptical, stationary bike, and walking with a weighted backpack containing 30% of body mass). Steady-state, aerobic-type exercise was chosen as the primary means by which total daily energy expenditure was elevated during phase 2 to reflect the aerobic-type physical work that occurs during sustained, strenuous military operations. On average, participants performed 3.5 exercise sessions per day. The physical activity prescription was verified biweekly and adjusted as needed to achieve the desired energy expenditure using open circuit indirect calorimetry (TruOne 2400, ParvoMedics, Salt Lake City, UT). Light calisthenics were incorporated into the exercise routine every 3 to 4 days. Adherence to the prescribed exercise was ≥89% and not different between groups.

Phase 3 (days 43–56) was a free-living, ad libitum recovery period. Participants were instructed to return to their prestudy diet and physical activity routines. Average iron content of the diets during phase 3 were 19.3 ± 7.0 mg/day and 31.4 ± 24.7 mg/day for the placebo and testosterone groups, respectively (P = 0.03).

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Submaximal exercise test

To assess submaximal aerobic adaptations in response to testosterone and energy deficit, participants performed 60 minutes of steady-state aerobic exercise on a cycle ergometer on days 14 and 42. Exercise intensity was matched within participants based on power output and total work performed to limit the confounding effects of weight loss on relative exercise intensity.

Blood collection

Fasting blood samples were collected on days 0, 14, 42, and 56 between 6:00 AM and 9:00 AM. Total testosterone, estradiol, and serum ferritin were measured using an automated immunoassay instrument (Siemens Medical Solutions USA Inc., Malvern, PA). Free testosterone was calculated (16). Hemoglobin was measured using the Beckman Coulter DxH (Beckman Coulter, Brea, CA). Hematocrit was calculated by multiplying red blood cell count by mean cell volume. Erythropoietin and soluble transferrin receptor (sTfR) (R&D Systems Inc., Minneapolis, MN), serum hepcidin (high-sensitivity, DRG International, Springfield, NJ) and erythroferrone (ERFE) (Instrinsic Life Sciences, La Jolla, CA) were determined using enzyme-linked immunoassays. Interleukin-6 (IL-6) was determined using a kit (Millipore, Burlington, MA) and read on a Luminex instrument. Intra- and interassay coefficient of variation for enzyme-linked immunoassays were EPO (5.2% and 8.3%), sTfR (6.4% and 7.1%), hepcidin (4.1% and 14.4%), ERFE (11.0% and 15.0%), and IL-6 (5.0% and 20.0%), respectively. All assays were conducted by Pennington Biomedical Research Center. Pennington Biomedical Research Center follows good clinical practices and is accredited by the College of American Pathologists. All assays were run with standards and appropriate quality control material. In addition, Pennington Biomedical Research Center runs external proficiency samples and results are compared with other laboratories across the country.

Statistical analysis

Data were analyzed using SAS, version 9.4. Differences at baseline (day 0) were analyzed using Student's t-tests. Time-by-treatment effects were analyzed using linear mixed models adjusted for age and baseline values. Bonferroni corrections were used for post hoc comparisons. $P < 0.05$ was considered statistically significant.

Results

Adjusted least square means for clinical biomarkers following each study phase are shown in Table 2. As reported previously (15), total and free testosterone concentrations increased by 134% and 155%, respectively, with testosterone administration during energy deficit, whereas concentrations remained stable or declined with placebo administration. Testosterone administration during energy deficit attenuated the decline in body weight that occurred with placebo.

Hepcidin concentrations declined by 41% with testosterone and remained stable with placebo during energy deficit (P-interaction = 0.0003). Serum ferritin

Table 2. Adjusted least square means for clinical biomarkers for placebo and testosterone following each study phase

	Placebo (n = 26)			Testosterone (n = 24)				P		
	Day 14	Day 42	Day 56	Day 14	Day 42	Day 56	Treatment	Day	Interaction
Weight, kg	78.3 (77.7, 78.9)[a]	73.3 (72.7, 74.0)[b]	76.5 (75.8, 77.1)[c]	78.0 (77.4, 78.7)[a]	75.8 (75.1, 76.5)[b*]	79.3 (78.6, 79.9)[a*]	<0.0001	<0.0001	<0.0001
TT, nmol/L	14.6 (12.9, 16.4)[a]	11.8 (10.0, 13.5)[a]	14.6 (12.8, 16.3)[a]	15.5 (13.7, 17.4)[a]	36.2 (34.3, 38.0)[b*]	7.8 (5.9, 9.8)[c*]	<0.001	<0.001	<0.0001
FT, nmol/L	0.31 (0.26, 0.36)[a]	0.18 (0.13, 0.23)[b]	0.29 (0.23, 0.34)[a,b]	0.33 (0.27, 0.38)[a]	0.87 (0.81, 0.92)[b*]	0.15 (0.10, 0.21)[c*]	<0.001	<0.001	<0.0001
Estradiol, pmol/L	142 (124, 159)[a]	131 (114, 148)[a]	158 (141, 175)[a]	148 (130, 166)[a]	247 (229, 265)[b*]	144 (125, 162)[a]	<0.001	<0.001	<0.0001
Hemoglobin, g/L	144 (142, 147)[a]	138 (136, 141)[b]	139 (137, 142)[b]	145 (143, 148)[a]	145 (142, 148)[a*]	145 (142, 147)[a]	0.002	0.012	0.035
Hematocrit, %	42.8 (42.0, 43.6)[a]	41.5 (40.7, 42.3)[a]	41.5 (40.7, 42.3)[a]	42.9 (42.1, 43.7)[a]	43.6 (42.7, 44.4)[a*]	43.2 (42.3, 44.0)[a]	0.003	0.450	0.020
EPO, mIU/mL	8.8 (7.6, 9.9)[a]	8.5 (7.1, 9.8)[a]	14.1 (12.0, 16.2)[b]	8.4 (7.2, 9.6)[a]	11.3 (9.9, 12.8)[b]	8.8 (6.5, 11.1)[a,b*]	0.218	0.0003	0.0002
Hepcidin, ng/mL	8.1 (6.6, 9.8)[a,b]	10.1 (8.5, 11.6)[a]	6.7 (5.1, 8.3)[b]	8.6 (7.0, 10.3)[a]	5.1 (3.4, 6.7)[b*]	6.4 (4.7, 8.2)[a,b]	0.044	0.076	0.0003
Ferritin, ng/mL	92.6 (78.7, 106.5)[a,b]	107.9 (94.2, 121.6)[a]	76.3 (62.6, 90.0)[b]	91.0 (76.8, 105.2)[a]	59.1 (44.6, 73.5)[b*]	47.2 (32.1, 62.2)[b*]	0.001	<0.0001	0.0001
sTfR, nmol/L	17.0 (15.4, 18.6)[a]	18.3 (16.7, 19.9)[a,b]	20.6 (19.1, 22.3)[b]	17.7 (16.1, 19.4)[a]	20.6 (18.9, 22.2)[b]	24.4 (22.6, 26.1)[c*]	0.021	<0.0001	0.139
sTfR/$\log_{10}$ ferritin	9.5 (8.9, 10.1)[a]	10.1 (8.8, 11.5)[a,b]	11.8 (10.1, 13.6)[b]	9.7 (9.1, 10.3)[a]	12.5 (11.1, 13.9)[b]	15.5 (13.6, 17.5)[c]	0.005	<0.0001	0.003
IL-6, pg/mL	8.5 (6.2, 10.8)	10.2 (7.9, 12.5)	11.1 (8.9, 13.4)	9.2 (6.9, 11.6)	8.5 (6.1, 10.9)	11.9 (9.4, 14.5)	0.971	0.054	0.401
ERFE, ng/mL	3.0 (2.2, 3.9)	3.6 (2.8, 4.5)	2.9 (2.1, 3.8)	3.4 (2.6, 4.3)	3.0 (2.1, 3.8)	3.4 (2.5, 4.3)	0.860	0.953	0.165

Values are adjusted least square means (95% confidence interval). Time-by-treatment effects were analyzed using linear mixed models, adjusted for age and baseline values. Bonferroni corrections were used for post hoc comparisons. $P < 0.05$ was considered statistically significant. Data not sharing the same letter superscript within a treatment are different by day. Asterisk indicates a between group difference at a particular day.

Abbreviations: EPO, erythropoietin; ERFE, erythroferrone; FT, free testosterone; IL-6, interleukin-6; sTfR, soluble transferrin receptor; TT, total testosterone.

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concentrations declined by 35% (P-interaction = 0.0001) and sTfR increased by 16% (P-treatment = 0.021) with testosterone and was not affected with placebo during energy deficit. Likewise, the sTfR/$\log_{10}$ ferritin ratio, an index of iron-dependent erythropoietic activity, increased by ~30% with testosterone but remained unchanged with placebo during energy deficit (P-interaction = 0.0026).

Testosterone prevented the decline in hemoglobin (P-interaction = 0.035) and hematocrit (P-interaction = 0.020) that occurred with placebo during energy deficit, such that hemoglobin and hematocrit were 5% greater at the end of energy deficit with testosterone compared to placebo. Erythropoietin increased by 34% with testosterone during energy deficit, whereas energy deficit alone did not affect EPO concentrations (P-interaction = 0.0002). Erythroferrone and IL-6 were not affected by energy deficit or testosterone.

As reported previously, lower-body muscle function declined in both groups with energy deficit (15). Submaximal aerobic adaptations in response to testosterone and energy deficit are shown in Table 3. Total energy, heart rate, perceived exertion, and measures of respiratory gas exchange improved and there was a greater reliance on fat oxidation during phase 2 regardless of treatment.

Discussion

The majority of iron required to support erythropoiesis is provided by reticuloendothelial macrophages, which recycle approximately 20 mg to 25 mg iron per day from senescent red blood cells. Iron availability is regulated by the liver-produced hormone, hepcidin. Hepcidin binds to ferroportin, which is expressed by macrophages, enterocytes, and hepatocytes, signaling for its internalization and degradation, thereby limiting iron availability for erythropoiesis. In the current study, hepcidin concentrations were markedly suppressed with testosterone compared to placebo during energy deficit.

Concomitant with the decline in hepcidin was a decline in serum ferritin concentration and an increase in sTfR with testosterone compared to placebo during energy deficit. The transferrin receptor is shed from the plasma membrane of erythrocytes during maturation and circulates as sTfR. Thus, circulating sTfR concentrations are a measure of total erythroid activity and reflect plasma iron turnover and erythroid transferrin uptake if iron deficiency is not present (17). Along with the increase in sTfR/$\log_{10}$ ferritin ratio, an index of iron-dependent erythropoietic activity (17, 18), these findings suggest that suppressed hepcidin and a decline

Table 3. Exercise intensity, perceived exertion, respiratory gas exchange, and substrate oxidation during 60 minutes of steady-state aerobic exercise during phases 1 and 2

	Placebo (n = 26)		Testosterone (n = 24)		*P*		
	Day 14	Day 42	Day 14	Day 42	Treatment	Day	Interaction
Total work, kJ	451 (412, 490)	451 (412, 490)	462 (422, 502)	462 (421, 502)	0.89	0.70	0.58
Power output, W	125 (114, 136)	125 (114, 136)	128 (117, 139)	128 (117, 139)	0.81	0.70	0.40
Total energy, kcals	616 (571, 661)	540 (501, 579)	640 (593, 687)	573 (533, 614)	<0.0001	0.31	0.65
Heart rate, bpm	162 (157, 167)	131 (126, 136)	158 (153, 163)	131 (125, 136)	<0.0001	0.45	0.40
Rating of perceived exertion	15 (15, 16)	13 (13, 14)	15 (14, 16)	13 (12, 14)	<0.0001	0.80	0.98
Respiratory gas exchange							
VO_2, L/min	2.09 (1.93, 2.24)	1.88 (1.75, 2.01)	2.16 (2.00, 2.31)	1.98 (1.85, 2.12)	<0.0001	0.36	0.63
VO_2 peak, %	61.0 (58.8, 63.2)	55.2 (52.6, 57.8)	60.3 (58.0, 62.6)	55.7 (53.1, 58.4)	<0.0001	0.96	0.51
VCO_2, L/min	1.88 (1.74, 2.02)	1.48 (1.37, 1.60)	1.99 (1.84, 2.13)	1.61 (1.49, 1.73)	<0.0001	0.18	0.78
RER	0.90 (0.89, 0.91)	0.79 (0.77, 0.80)	0.92 (0.91, 0.93)	0.81 (0.79, 0.82)	<0.0001	0.01	0.96
Substrate oxidation, total g							
Carbohydrate, total g	104 (95, 114)	40 (32, 48)	119 (109, 129)	53 (45, 62)	<0.0001	0.01	0.81
Fat, total g	22.0 (18.7, 25.4)	42.1 (38.5, 45.7)	18.2 (14.7, 21.6)	40.0 (36.2, 43.7)	<0.0001	0.13	0.58

Values are adjusted least square means (95% confidence interval). Time-by-treatment effects were analyzed using linear mixed models and adjusted for age. Adapted from (15).
Abbreviations: RER, respiratory exchange ratio; VCO_2, volume of exhaled carbon dioxide; VO_2, volume of oxygen uptake.

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in functional iron stores support the increase in erythropoiesis with testosterone during energy deficit.

It is unclear whether testosterone directly suppresses hepcidin or indirectly suppresses hepcidin through its effects on EPO. Erythropoiesis is stimulated by EPO, a renal hormone that rapidly increases the expression of transferrin receptor on the plasma membrane of erythroid precursors. Transferrin receptor can then take up transferrin-bound iron in circulation to produce heme for incorporation into hemoglobin. During steady-state erythropoiesis in healthy human adults, this process translates to 20 mL of blood production each day, containing 6 g of hemoglobin and 20 mg of iron. A study in older men receiving 10 g testosterone gel or placebo daily demonstrated that testosterone stimulates the secretion of EPO (12). The response appears to be transient, as the increase in EPO was observed at 1 and 3 months but returned to placebo levels at 6 months. In the current study, EPO increased by 34% compared to baseline values with 1 month of testosterone administration during energy deficit, whereas energy deficit alone did not affect EPO concentrations. The increase in EPO with testosterone during energy deficit is consistent with animal studies that demonstrate an exponential increase in ^{59}Fe incorporation into erythrocytes in starved rats receiving graded doses of EPO (19).

Recent studies have demonstrated that EPO stimulates the secretion of ERFE from erythroid precursors (20). Erythropoietin-stimulated ERFE suppresses hepcidin levels, resulting in an increase in circulating levels of iron and iron incorporation into erythrocytes. However, in the current study, ERFE was not affected by energy deficit or testosterone, suggesting that the decrease in hepcidin with testosterone occurs through an ERFE-independent mechanism. Alternatively, a biphasic effect of EPO on hepcidin expression was recently proposed: an ERFE-independent repression in hepcidin where the immediate and transient decrease in serum iron with EPO inhibits the BMP-SMAD pathway and an ERFE-dependent repression whereby hepcidin is repressed until increased iron needs are met (21). Other possibilities include a direct effect of testosterone on bone morphogenetic protein (BMP)–sons of mother against decapentaplegic (SMAD) signaling (11) and/or the aromatization of testosterone to estradiol, which suppresses hepcidin transcription (22, 23). The lack of change in IL-6 during energy deficit in the current study excluded the possibility of a mediating role of the IL-6–induced Janus kinase (JAK)–signal transducer and activator of transcription 3 (STAT3) signaling pathway on hepcidin transcription.

Findings from the current study indicate that testosterone supplementation suppresses hepcidin in healthy human adults, thereby increasing iron utilization and maintaining erythropoiesis during energy deficit. Despite these findings, submaximal aerobic adaptations improved in both groups regardless of treatment. This is likely due to the aerobic-type exercise performed during phase 2 to increase total daily energy expenditure. Future work should investigate whether attenuations in weight loss and maintained erythropoiesis with testosterone supplementation during severe energy deficit lead to improvements in performance outcomes. Furthermore, rodent models may be necessary to determine the mechanism by which testosterone suppresses hepcidin during severe energy deficit. These findings suggest that testosterone administration may be an effective therapy to maintain erythropoiesis during energy deficit, which may be particularly relevant for military training or operations and in certain types of anemias of chronic disease (24, 25).

Acknowledgments

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or the Department of Defense. Any citations or commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement of approval of the products or services of these organizations. Supported by the Collaborative Research to Optimize Warfighter Nutrition II and III projects, the Defense Health Program Joint Program Committee-5, and appointment to the U.S. Army Research Institute of Environmental Medicine administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the U.S. Army Medical Research and Materiel Command.

Clinical Trial Information: Registered at www.clinicaltrials.gov as #NCT02734238.

Additional Information

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Disclosure Summary: The authors have nothing to disclose.

Data Availability: All data generated or analyzed during this study are included in this published article or in the data repositories listed in the References section.

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Bradbury et al. Journal of the International Society of Sports Nutrition (2020) 17:4
https://doi.org/10.1186/s12970-020-0335-2

Journal of the International Society of Sports Nutrition

SHORT REPORT

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Effects of carbohydrate supplementation on aerobic exercise performance during acute high altitude exposure and after 22 days of acclimatization and energy deficit

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Abstract

Background: The ergogenic effects of supplemental carbohydrate on aerobic exercise performance at high altitude (HA) may be modulated by acclimatization status. Longitudinal evaluation of potential performance benefits of carbohydrate supplementation in the same volunteers before and after acclimatization to HA have not been reported.

Purpose: This study examined how consuming carbohydrate affected 2-mile time trial performance in lowlanders at HA (4300 m) before and after acclimatization.

Methods: Fourteen unacclimatized men performed 80 min of metabolically-matched (~ 1.7 L/min) treadmill walking at sea level (SL), after ~ 5 h of acute HA exposure, and after 22 days of HA acclimatization and concomitant 40% energy deficit (chronic HA). Before, and every 20 min during walking, participants consumed either carbohydrate (CHO, $n = 8$; 65.25 g fructose + 79.75 g glucose, 1.8 g carbohydrate/min) or flavor-matched placebo (PLA, $n = 6$) beverages. A self-paced 2-mile treadmill time trial was performed immediately after completing the 80-min walk.

Results: There were no differences ($P > 0.05$) in time trial duration between CHO and PLA at SL, acute HA, or chronic HA. Time trial duration was longer ($P < 0.05$) at acute HA (mean ± SD; 27.3 ± 6.3 min) compared to chronic HA (23.6 ± 4.5 min) and SL (17.6 ± 3.6 min); however, time trial duration at chronic HA was still longer than SL ($P < 0.05$).

Conclusion: These data suggest that carbohydrate supplementation does not enhance aerobic exercise performance in lowlanders acutely exposed or acclimatized to HA.

Trial registration: NCT, NCT02731066, Registered March 292,016

Keywords: Hypoxia, Time trial, Ergogenic aid

Background

Reduced arterial oxygen content degrades aerobic exercise performance in lowlanders initially exposed to high altitude (HA) [1]. After several weeks of HA exposure, acclimatization occurs, and aerobic exercise performance partially improves [1–3]. Another factor that may impact the performance degradation seen at altitude are changes in substrate oxidation [4], as endogenous carbohydrate oxidation during exercise may be higher with acute HA exposure compared to absolute VO_2-matched exercise at sea level (SL) [5]. After acclimatization, muscle glucose uptake during exercise is also increased relative to SL [6]. Collectively, these data suggest that consuming supplemental carbohydrate during exercise at HA might be an effective strategy to attenuate performance decrements by sparing endogenous carbohydrate stores and meeting the apparent increase in carbohydrate requirements during exercise.

Carbohydrate supplementation during exercise at SL spares endogenous carbohydrate stores and delays the onset of fatigue [7, 8]. Effects of carbohydrate supplementation on

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exercise performance at HA are not well described. In unacclimatized men, Fulco et al. [9] demonstrated that carbohydrate supplementation during exercise enhanced time trial performance compared to placebo after 3 days of HA (4300 m) exposure concomitant with a 30% energy deficit (negative energy balance is largely unavoidable and commonly experienced by lowlanders sojourning at HA) [10]. However, carbohydrate supplementation had no further performance benefit after 10 days of acclimatization and negative energy balance. In a follow-up study, Fulco et al. [11] found no benefit of carbohydrate supplementation on time trial performance on the first and third day of HA exposure in previously HA acclimatized men in a state of energy balance. These discordant results from vastly different experimental designs (i.e., energy and acclimatization status of participants) suggest that the potential ergogenic effects of supplemental carbohydrate on exercise performance at HA may, in part, be modulated by acclimatization. To extend these findings [9, 11], we examined the effects of carbohydrate supplementation on aerobic exercise performance in unacclimatized men after 5 h of HA (4300 m) exposure and following 22 days of HA acclimatization and concomitant 40% energy deficit. Based on the studies by Fulco and colleagues [9, 11], we hypothesized that carbohydrate supplementation would enhance exercise performance during acute HA exposure, but not after HA acclimatization.

Methods

Experimental design

Data included in this Short Report were secondary analyses from a controlled feeding and exercise study that assessed the effects of high protein diets on body composition during sustained energy deficit at HA [12]. This study (clinical trials.gov: NCT02731066) was approved by the Institutional Review Board at the US Army Research Institute of Environmental Medicine (USARIEM, Natick, MA) and participants provided written informed consent. Characteristics of the 14 unacclimatized, young men included in this report were detailed by Young et al. [13].

The experimental design has been reported extensively [12–16]. In brief, the study was conducted over 43 consecutive days. During the first 21 days (SL), participants consumed a self-selected, weight-maintaining diet, maintained habitual exercise levels, and were free-living but visited the laboratory daily. On SL day 21, participants were flown from Boston, MA to Denver, CO where they were placed on supplemental oxygen until being driven to the summit of Pikes Peak, CO (4300 m) the following morning where they resided at the USARIEM Maher Memorial Altitude Laboratory for the next 22 days at HA. During HA, participants were under constant supervision, performed daily exercise, and consumed either standard protein (mean ± SD; 1.1 ± 0.2 g/kg/d) or high protein (2.1 ± 0.2 g/kg/d), carbohydrate-matched, energy deficient diets (40%; 30% by energy restriction and 10% by exercise). Fat was the primary manipulated macronutrient during the energy deficit, such that the standard protein group consumed 1.1 ± 0.2 g/kg/d fat, and the high protein group consumed 0.7 ± 0.1 g/kg/d fat [12]. The diet intervention resulted in a 7.9 ± 1.9 kg loss of total body mass [13].

Exercise and 2-mile time trial

Participants were randomized to groups and were provided equal volumes of flavor-matched carbohydrate (CHO; 65.25 g fructose + 79.75 g glucose ingested at 1.8 g/min, $n = 8$, 3 standard and 5 high protein) and placebo (PLA; $n = 6$, 4 standard and 2 high protein) beverages during 80 min of metabolically-matched, steady-state treadmill walking at SL (day 7), 5 h after arriving at HA (acute HA), and after 22 days of acclimatization and energy deficit (chronic HA, day 42). As previously reported [13] treadmill speed, grade, absolute oxygen uptake (L/min), and metabolic rate were not different across study phases. All time trials were completed at the same time of day (between 1130 and 1230) and participants were fasted prior to the start of exercise. Participants were given a 5 min rest after completing the steady-state exercise before performing a self-paced, 2-mile treadmill time trial. Peripheral oxygen saturation (SpO_2; finger pulse oximetry, Model 9560; Nonin, Plymouth, MN, USA) and heart rate were assessed at baseline and in half mile intervals, and Ratings of Perceived Exertion (RPE) [17] were recorded at baseline and immediately after completing the 2-mile time trial. Participants were familiarized to the test procedures (80 min steady-state and 2-mile time trial) on three occasions before completing the SL trial on day 7. The coefficient of variation of the three familiarization time trials was 5.06%, indicating that the test-retest variation in performance was small.

Statistical analyses

As mentioned, this study was part of a larger investigation [12], powered to test the effects of dietary protein on body composition during altitude acclimatization and concomitant energy deficit. We did not expect dietary protein level to affect time trial performance at chronic HA (neither SL or acute HA were subject to the dietary protein intervention), which we confirmed using linear mixed models with dietary protein level, treatment (CHO, PLA), phase (SL, acute and chronic HA), exercise time point (for SpO_2 and heart rate), and their interactions as fixed effects. Therefore, the effects of the dietary protein intervention are not presented in this report, and the data were re-analyzed using the same linear mixed-model without dietary protein in the model. Tukey's HSD tests were used for multiple comparisons if significant main effects of interaction effects were

observed. Freidman's test was used to assess differences in RPE (baseline, end of the time trial) across study phases. Statistical significance was accepted at $P < 0.05$. Data were analyzed using SPSS (v.22.0; Chicago, IL, USA).

Results

There was no effect ($P > 0.05$) of CHO on time trial duration, oxygen saturation, heart rate, or RPE across study phases. Time trial duration was longer ($P < 0.05$) at acute HA (27.3 ± 6.3 min) compared to chronic HA (23.6 ± 4.5 min) and SL (17.6 ± 3.6 min); however, time trial duration at chronic HA was still longer than SL (phase main effect, $P < 0.05$, Fig. 1). Mean heart rate and SpO_2 during the time trials were lower at acute and chronic HA compared to SL, and SpO_2 at chronic HA was higher than acute HA (phase main effect, $P < 0.05$, Table 1). RPE was similar across study phases.

Discussion

This study examined the effects of consuming supplemental carbohydrate on aerobic exercise performance in recreationally-active, healthy, young lowlanders at HA, before and following 22 days of acclimatization while in a constant state of negative energy balance. We demonstrated that consuming supplemental carbohydrate during steady-state exercise had no performance-enhancing effects on time trial performance before or after HA acclimatization. As expected, time trial performance was approximately 55% slower at acute HA compared to SL. Acclimatization partially restored exercise performance relative to acute HA; however, time trial performance at chronic HA was still 34% slower than SL. Carbohydrate supplementation had no effect on perceived effort during any of the performance trials. These data, which were derived from a highly controlled exercise and diet intervention study, suggest that carbohydrate supplementation does not enhance aerobic exercise performance of lowlanders at HA.

In our study, supplemental carbohydrate did not enhance exercise performance in lowlanders acutely exposed to HA (5 h), which conflicts with data reported by Fulco et al. [9], who demonstrated that lowlanders consuming carbohydrate on the third day of continuous residence at 4300 m performed a 720 kJ cycle ergometry time trial faster than participants consuming the placebo. We suspect the discrepancy between our data and those by Fulco et al. [9] are due, in part, to the apparent inability to effectively oxidize exogenous carbohydrate upon initial exposure to HA. In the same study, we demonstrated a 52% reduction in exogenous carbohydrate oxidation and a corresponding increase in endogenous carbohydrate oxidation (i.e., blood glucose, muscle and liver glycogen) during the 80 min steady-state exercise bout with acute HA exposure compared to SL[13]. The mechanisms accounting for the reduction in exogenous carbohydrate oxidation during acute HA exposure are not known, but may be attributable to adjustments in glycemic regulation, as participants were markedly hyperinsulinemic, which inhibited lipolysis and likely accelerated glycogenic flux. The inability to effectively oxidize exogenous carbohydrate during acute HA exposure was alleviated after 22 days of acclimatization [13]. It is possible that the adaptations that improve exogenous carbohydrate oxidation with acclimatization develop within only a few days of HA exposure, which would account for the performance enhancing effects observed by Fulco et al. [9] after 3 days of acclimatization. That, combined with the longer duration time trial (greater reliance on carbohydrates) in the Fulco study [9] (720 kJ cycle ergometry time trial), compared to our 2-mile time trial, may explain why they observed improvements in time trial performance and we did not.

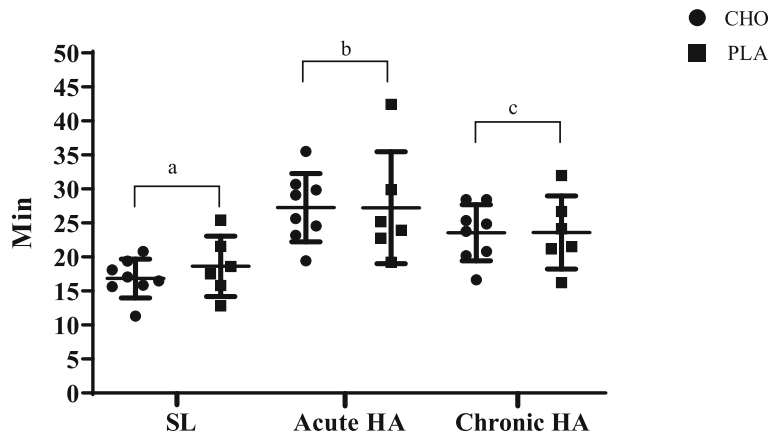

Fig. 1 2-mile time trial duration (min) at sea level, acute and chronic HA for CHO and PLA. Values are presented as mean ± SD. Points not sharing the same letter are different (phase main effect, P < 0.05). SL, sea level; HA, high altitude; CHO, carbohydrate; PLA, placebo

Table 1 Heart rate, peripheral oxygen saturation, and ratings of perceived exertion during the 2-mile time trial[a]

	SL		Acute HA		Chronic HA	
	CHO	PLA	CHO	PLA	CHO	PLA
HR (bpm)[2]	181 ± 10	175 ± 9	159 ± 9	168 ± 14	166 ± 13	161 ± 18
SpO_2 (%)[2,3]	93 ± 2	95 ± 3	71 ± 4	73 ± 4	77 ± 3	81 ± 4
RPE	17 (14–19)	17 (16–18)	16 (13–20)	18 (15–20)	18 (14–19)	19 (16–19)

[a]Values presented as mean ± SD, except for RPE which is presented as median (range)
[2]Phase main effect; acute and chronic HA different than SL, $P < 0.05$
[3]Phase main effect; chronic HA different than acute HA, $P < 0.05$.
SL Sea level, *HA* High altitude, *HR* Heart rate, *CHO* Carbohydrate, *PLA* Placebo, *SpO_2* Peripheral oxygen saturation, *RPE* Rating of Perceived Exertion

Our chronic HA results are in line with what has been previously reported about the influence of carbohydrate supplementation on time trial performance after acclimatization [9, 11]. Although we demonstrated that ability to oxidize exogenous carbohydrates was restored with acclimatization to HA [13], carbohydrate supplementation still did not improve performance. However, exercise performance in both carbohydrate and placebo following 22 days of acclimatization and energy deficit did improve relative to acute HA as a result of the normal adaptive responses that occur with acclimatization. Since muscle glycogen utilization is related to relative exercise intensity (%VO_{2peak}) [9, 11, 18], it may be that the exercise intensity during the time trials was not high enough to cause decreases in muscle glycogen that would cause the body to rely on exogenous sources of carbohydrate to complete the exercise task.

The practical implications of our findings must be interpreted in the context of certain experimental limitations. For example, it may be that carbohydrate supplementation did not improve performance due to the potential that such high carbohydrate intakes, particularly at altitude, may have reduced gastric emptying and intestinal carbohydrate absorption [19]. However, those measures were not included in our study. Likewise, our applied performance outcomes may have been strengthened if additional mechanistic measures oxygen transport capacity were included. Our design also does not allow us to completely rule out the potential effects of sustained energy deficit on time trial performance after 22 days of acclimatization. However, including fully fed, energy balance control groups was impractical and outside the scope of our study. We also contend that our findings, which show that carbohydrate supplementation failed to enhance performance following 22 days of acclimatization and concomitant energy deficit, are not only consistent with Fulco et al. [9], but are actually strengthened by our experimental design. More specifically, rather than experimentally enforcing energy balance, we assessed the putative performance enhancing effect of supplemental carbohydrate in response to the real world conditions (i.e., negative energy balance) that lowlanders typically experience during prolonged HA sojourns [10]. Most importantly, considering carbohydrate supplementation did not affect performance, and reliance on endogenous carbohydrate to fuel steady-state exercise appears to be higher during acute HA exposure than it is for absolute intensity-matched exercise at sea level [20], provides practical evidence to recommend that individuals planning to sojourn at HA to complete physically demanding work or exercise should prioritize carbohydrate intake at SL to optimize glycogen stores before ascending to HA.

Conclusion

We conclude that carbohydrate supplementation during steady-state exercise does not enhance exercise performance in lowlanders acutely exposed to hypoxia or sojourning at HA for 22 days.

Abbreviations

CHO: Carbohydrate; HA: High altitude; PLA: Placebo; RPE: Rating of Perceived Exertion; SL: Sea level; SpO_2: Oxygen saturation; USARIEM: US Army Research Institute of Environmental Medicine

Acknowledgements

We thank the volunteers that participated in the study. We acknowledge our medical oversight team and Dr. Stephen Muza for their support of this study. Importantly, we sincerely thank Mr. Bradley Anderson, Ms. Katelyn Guerriere, Mr. Grant Holmes, Mr. Anthony Karis, Mr. Matthew Kominsky, Ms. Allyson Derosier, Ms. Adrienne Hatch, Ms. Laura Lutz, Mr. Phillip Niro, Ms. Nancy Murphy, Drs. Steven Hennigar, Taylor Roth, Arny Ferrando, J. Phillip Karl, Lee Margolis, Scott Montain, John Carbone, James McClung, and LTC Renee Cole for their significant contributions to the study.

Declarations

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or the Department of Defense. Any citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement of approval of the products or services of these organizations.

Authors' contributions

CEB, RWK, AJY, and SMP designed the research; KEB, CEB, MAW, AJL, RWK, AJY, and SMP performed the research. KEB and AJL analyzed the data. KEB and SMP prepared tables, figures, and drafted the manuscript. All authors edited and revised the manuscript and approved the final version.

Funding

This work was supported by the US Army Medical Research and Development Command.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
The study was approved by the Institutional Review Board at the US Army Research Institute of Environmental Medicine (USARIEM, Natick, MA) and participants provided written informed consent prior to any participation in the study. The research was performed in accordance with the Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 9 October 2019 Accepted: 3 January 2020
Published online: 09 January 2020

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Eating Behaviors 36 (2020) 101367

Contents lists available at ScienceDirect

Eating Behaviors

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Body weight status, perceived stress, and emotional eating among US Army Soldiers: A mediator model

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ARTICLE INFO

Keywords:
Stress
Emotional eating
Body mass index
Body composition
Military
Mediation analysis

ABSTRACT

Background: In civilians, overweight and obesity are associated with emotional eating behaviors such as eating in response to stress, but this association has not been examined in Soldiers, a population with unique stressors. This cross-sectional study examined the relationship between perceived stress (PS), emotional eating (EE), and outcomes of body mass index (BMI), and failing Army body composition (BC) standards among Soldiers.
Methods: Soldiers (N = 1460, 83% male, 23.5 ± 5.2 years old) completed validated surveys on PS, EE, and adherence with military BC standards. Conditional process models and mediation models tested gender as a moderator and EE as a mediator of associations between PS and BMI and PS and BC failure, respectively.
Results: Higher PS was associated with more frequent self-reported EE behaviors ($p < 0.001$), higher BMI ($p < 0.001$), and BC failure ($p < 0.001$). BMI significantly increased with frequency of reported EE behaviors ($p < 0.001$). Gender was not a statistically significant moderator in the relationship between PS, EE, and, BMI ($p = 0.83$) or BC failure ($p = 0.57$). PS appears to affect BMI indirectly through EE behaviors ($c' = 0.03$, 95% CI: 0.02, 0.04). PS may affect BC failure directly ($c' = 1.04$, 95% CI: 1.01, 1.08) and indirectly ($ab = 1.02$, 95% CI: 1.01, 1.03) through EE as a mediator.
Conclusions: EE behaviors may mediate the positive association between PS, BMI, and BC failure. Prospective investigation is warranted to better understand the role of EE in health-related outcomes among Soldiers and populations in high stress professions.

1. Introduction

Overweight and obesity among Soldiers is a major concern for the US Army. Although Soldiers are required to meet body weight and body composition standards, the prevalence of overweight and obesity has risen such that in 2017, over 17% were considered obese based on a body mass index (BMI) of $\geq 30\,kg/m^2$ (https://phc.amedd.army.mil/topics/campaigns/hof/Pages/default.aspx, 2017). Soldiers with a higher BMI have an increased risk of injury and illness (Cowan, Bedno, Urban, Yi, & Niebuhr, 2011; Hruby, Bulathsinhala, McKinnon, et al., 2016; Knapik, Jones, Darakjy, et al., 2007), which can limit their ability to meet job requirements and causes a significant financial burden to the military health care system (Dall, Zhang, Chen, et al., 2007). Understanding the underlying factors contributing to overweight and obesity among Soldiers is critical to informing the development of healthy weight management interventions and, ultimately, improving military readiness (Hauret, Jones, Bullock, Canham-Chervak, & Canada, 2010; McNulty, 2009).

One potential underlying factor is stress. Similar to other public service professionals (e.g. first responders), Soldiers commit to a highly stressful and often hazardous lifestyle, and must deal with unique military-specific stressors such as physically demanding job duties, upheavals resulting from deployments, and routine relocations. Stress negatively impacts health through psychological, physiological, and behavioral pathways (Hammen, 2005; Steptoe, 1991), and is known to contribute to a variety of health issues, including overweight/obesity (Lagraauw, Kuiper, & Bot, 2015). For example, in civilian populations, higher stress levels have been associated with elevated BMI (Laugero,

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https://doi.org/10.1016/j.eatbeh.2020.101367
Received 1 May 2019; Received in revised form 13 January 2020; Accepted 14 January 2020
Available online 16 January 2020
1471-0153/ Published by Elsevier Ltd.

Falcon, & Tucker, 2011; Mouchacca, Abbott, & Ball, 2013), increased visceral adipose tissue (Adam & Epel, 2007; Mouchacca et al., 2013; Nastaskin & Fiocco, 2015), and weight gain (Torres & Nowson, 2007).

Relationships between stress and overweight/obesity are thought to be mediated, in part, by the influence of stress on eating behaviors (Koenders & van Strien, 2011; Wiedemann, Ivezaj, & Barnes, 2018). For example, stress has been associated with increased energy intake due to higher consumption of palatable foods, specifically fats and sugars (Born et al., 2011; Laugero et al., 2011; Nastaskin & Fiocco, 2015). Research in civilian populations has shown that individuals overeat to reduce stress, anxiety, fear, or negative emotions (Greeno & Wing, 1994), and eating in response to emotional stimuli such as stress, or emotional eating (EE), has been associated with weight gain (Bongers & Jansen, 2016). Further, stress alone has been associated with increased energy intake due to higher consumption of palatable foods, specifically fats and sugars (Born et al., 2011; Greeno & Wing, 1994; Laugero et al., 2011; Nastaskin & Fiocco, 2015).

In civilian studies, variations in the extent to which eating behaviors are affected by stress have been observed. For example, EE appears to be more prevalent among women, who may choose higher fat and sugar foods when under stress than men (Adam & Epel, 2007; Peneau, Menard, Mejean, Bellisle, & Hercberg, 2013). Women, who often have additional sources of stress than men as primary caregivers and caretakers (Wahl & Rundall, 1996), are more prone to increase food consumption when under stress (Beydoun, 2014; Nevanpera et al., 2012; Peneau et al., 2013). Eating in response to emotional stimuli such as stress may therefore contribute to overweight and obesity. The effect of EE may be stronger among populations in high stress professions and may be of greater concern among women in these professions.

While stress inherent to military or public service is to some extent unavoidable, an improved understanding of the relationship between stress and undesirable health outcomes, such as overweight and obesity, is needed to inform the development of interventions aimed at minimizing the adverse health effects of stress. To date, no study has examined the extent to which EE mediates associations between stress and body weight outcomes in Soldiers. Therefore, this cross-sectional study in Soldiers examined gender as a moderator and EE as a mediator of associations between perceived stress (PS), BMI, and failing body composition (BC) standards, respectively. We hypothesized that, in Soldiers, frequency of EE behaviors would be greater among female Soldiers, and would mediate associations between PS and BMI and PS and BC failure, respectively.

2. Methods

2.1. Study population

From 2016 to 2017, 1544 Soldiers from Army installations at Fort Jackson, SC, Joint-Base Lewis McChord, WA, Fort Riley, KS, Fort Sam Houston, TX, Fort Carson, CO, Joint Base Elmendorf-Richardson, AK, and Fort Campbell, KY were briefed in person and a total of 1460 (95%) completed surveys. Soldiers completing the survey ranged in age from 18 to 58 years (mean ± SD 23.5 ± 5.2). Soldiers were surveyed to obtain information on multiple topics conceptually related to eating behaviors or mediators of eating behaviors to include PS, EE, adherence with Army body composition (BC) standards, and sociodemographic information (military status, i.e., initial trainee or not a trainee), age, race/ethnicity, level of education, marital status, use of tobacco, and physical activity. All active-duty Soldiers 18 years of age and older were eligible to participate. A *post-hoc* power analysis using G*Power was conducted for regression models (Faul, Erdfelder, Lang, & Buchner, 2007). The sample size was more than sufficient for regression models with ten predictors to detect an R^2 as small as 0.02 or an odds ratio of 1.2 with 80% probability at a significance level of 0.05. The study was approved by the Institutional Review Board of the US Army Medical Research and Development Command, Fort Detrick, Maryland. Investigators adhered to the policies regarding the protection of human subjects as prescribed in Army Regulation 70–25, the research was conducted in adherence with the provisions of 32 CFR Part 219, all participants provided written informed consent prior to participation, and all participants were assured that their responses would be kept confidential.

2.2. Dependent variables

2.2.1. Body mass index and body composition failure

BMI was calculated from self-reported height and weight. Data were screened for biologically implausible values and outliers. Visual inspection and residual statistics during assumption testing yielded two BMI measures above the 99th percentile with a residual > 3. These two observations were demonstrated to be influential outliers when removing these observations significantly changed the estimate of coefficients. The Army assesses compliance with body composition standards twice yearly through BMI-based screening, and for those who exceed the maximum allowable screening weight, a circumference-based body fat assessment commonly referred to as the "tape test" is conducted (https://armypubs.army.mil/epubs/DR_pubs/DR_a/pdf/web/ARN7779_AR600-9_FINAL.pdf, 2019). Female Soldiers are measured at three circumference sites: neck, waist, hip, and male Soldiers are measured at two sites: neck and waist (https://armypubs.army.mil/epubs/DR_pubs/DR_a/pdf/web/ARN7779_AR600-9_FINAL.pdf, 2019). Soldiers who exceed maximum allowable body fat percent are counseled and provided with nutrition education (https://armypubs.army.mil/epubs/DR_pubs/DR_a/pdf/web/ARN7779_AR600-9_FINAL.pdf, 2019). In this study, BC failure was determined by the question "Have you ever failed the body fat tape test?" with possible responses being "yes" or "no".

2.3. Independent variables

2.3.1. Perceived stress

PS scores were assessed using Cohen's ten-item Perceived Stress Scale (PSS-10). The PSS-10 is a validated measure ($\alpha = 0.91$) (Cohen & Janicki-Deverts, 2012) where respondents rate how unpredictable, uncontrollable, or overloaded they perceive their life has been in the past 30 days on a 5-point Likert-type scale (Cohen, 1988). The PSS-10 has been used widely in diverse populations since its inception (Cohen & Janicki-Deverts, 2012). Each Soldier's PS score was based on the sum of their responses with a possible range of 0–40. Scores ranging from 0 to 13 are considered low perceived stress. Scores ranging from 14 to 26 are considered moderate perceived stress. Scores ranging from 27 to 40 are considered high perceived stress (Cohen, 1988).

2.4. Mediator

2.4.1. Emotional eating

EE was assessed by two questions taken from the Intuitive Eating Scale-2 (IES-2): "I found myself eating when I felt emotional (e.g., anxious, depressed, sad), even when I was not physically hungry" and "I found myself eating when I was stressed out, even when I was not physically hungry" on a 5-point Likert-type scale. The IES-2 is a validated tool ($\alpha = 0.87$) measuring tendency towards following internal cues guiding eating behaviors (Tylka & Kroon Van Diest, 2013). These questions from the IES-2 were chosen because they appeared to represent eating in response to external cues, such as in response to stress. The IES-2 prompts ask about behaviors in the present tense, but in this study, the response prompt was changed to ask about the last 30 days to match the timeframe of the PSS-10. Responses to these questions were combined to create a variable that grouped frequency of EE behaviors responses into three categories: "never" for those who selected never or rarely, "sometimes" for those who selected sometimes, or "often" for those who selected often or always.

2.5. Statistical analysis

Data were analyzed using Stata, v.15.1 (StataCorp, College Station, TX) or SPSS, v.26 (IMB Statistics). There was missing data among 72 (4.9%) Soldiers, therefore data was screened for patterns in missing data among variables. For the main variables of interest, 10 Soldiers had missing PS responses (0.7%), there were 39 missing EE responses (2.7%), 9 missing BMI responses (0.6%), and 13 missing BC failure responses (0.9%). No difference in the distribution of the variables between missing and non-missing was found. The data were also screened for violations of assumptions prior to analysis. BMI skewness and kurtosis was 0.32 and 2.84, respectively. Preliminary models using the natural log of BMI did not appreciably change model outcomes, therefore to aid interpretation, BMI was used without transformation (Jayne, Blake, Frongillo, et al., 2019). No other violations were found. Cronbach's alpha was used to assess scale reliability. The characteristics of Soldiers by frequency of EE behaviors was examined using chi square and ANOVA statistics to include post-hoc tests to show between group differences.

Linear regression analysis was implemented using the Stata *sem* command with full-information maximum likelihood estimation using the *mlvm* option to examine the effect of frequency of emotional eating behaviors on BMI. This estimation procedure assumes missing data among independent variables is missing at random, which makes use of all participants not missing on the dependent variable. Logistic regression analysis using the Stata *gsem* command was implemented for BC failure. Soldiers who had not been in the Army long enough to have been screened for BC were excluded from logistic regression models.

A conditional process model (also known as moderated mediation) as described by Hayes (Hayes, 2018) was implemented using the *process* command in SPSS to test the relationship between PS and BMI, also known as the direct effect, and examine if EE acts as a mediator between PS and BMI, also known as the indirect effect, with gender as a moderator. An additional model was implemented to test the same moderator-mediator relationships with the outcome of BC failure. The *process* command assumes complete data and excludes participants that have missing data on any of the variables included in the models. To evaluate gender as a moderator, interaction terms were examined for significance. Based on the moderation results, a simple mediation model, also implemented with the *process* command in SPSS, was generated for both outcomes of BMI and BC failure as either a multivariate linear regression or a logistic regression for the binary outcome of BC failure (Hayes, 2019). All models controlled for a Soldiers' military status (initial trainee or not a trainee), age, race/ethnicity, level of education, marital status, tobacco use, and physical activity. Training status was controlled for because Soldiers in training status have less choice in eating behaviors. Age was included as a control variable because of the association between BMI and age. Race/ethnicity, education level, and marital status were used as control variables to reduce confounding between the independent and dependent variables. We controlled for tobacco use since tobacco is often used for stress relief and for physical activity because of its relationship to BMI. Bootstrap confidence intervals were generated based on 10,000 bootstrap samples ensuring the normality of the sampling distribution and to assess the indirect effect of PS on BMI and BC, through EE. EE was considered to be a mediator if the bootstrap 95% CI for the indirect effect was above zero.

3. Results

Reliability testing of the PSS-10 ($\alpha = 0.93$) and IES-2 items ($\alpha = 0.91$) showed good internal consistency among the sample population. BMI was significantly different based on frequency of reported EE behaviors (Table 1). Among those who failed BC standards, a greater proportion reported more frequent EE behaviors. Compared to those who never reported EE behaviors, Soldiers who reported EE behaviors "often" also reported higher PS levels. Mean ± SD PS score was 17.5 ± 4.7 and 17.7 ± 4.4 for men and women, respectively ($p = 0.46$) (Cohen, 1988). These scores are higher than those measured in a similar aged cohort of 18–25 year old civilians (mean ± SD PS of 16.8 ± 6.8; $p = 0.02$) (Cohen & Janicki-Deverts, 2012). Gender differences in Soldiers reporting EE behaviors sometimes or often were significantly different.

Table 1
Characteristics of soldiers by frequency of emotional eating behaviors (N = 1460).

Sociodemographic variables, mean (SD) or %	Self-reported emotional eating behavior			
	Never	Sometimes	Often	*p*
Body mass index (kg/m^2)	25.5(4.5)	**25.8(3.7)**	**26.6(4.1)**	< 0.001
Failed body composition standards	14.5	**20.2**	**29.1**	< 0.001
Mean perceived stress score	16.7(4.5)	**18.6(4.3)**	**20.1(4.6)**	< 0.001
Perceived stress level				
Low (score: 0–13)	85.7	**9.4**	**4.9**	< 0.001
Moderate (score: 14–26)	65.3	**22.3**	**12.4**	< 0.001
High (score: 27–40)	36.1	36.1	**27.8**	< 0.001
Gender				
Male	70.7	**18.9**	**10.4**	< 0.001
Female	56.4	**26.9**	**16.7**	< 0.001
Age, years	23.5(5.2)	23.3(4.7)	24.1(6.1)	0.25
Rank categories				0.45
Enlisted	68.8	20.0	11.2	
Officer	62.6	23.1	14.3	
Marital status				0.31
Single	67.2	21.4	11.4	
Married	70.5	18.1	11.4	
Education level				0.03
High school/GED	71.5	19.2	**9.3**	
Some college	66.9	19.7	13.4	
Degree	61.4	25.5	13.1	
Race/ethnicity				0.17
Non-Hispanic White	67.5	21.5	11.0	
Non-Hispanic Black	71.3	19.4	9.3	
Non-Hispanic other	66.0	23.6	10.4	
Hispanic	55.1	17.1	27.8	
Recruit status				0.18
Trainee	63.9	23.4	12.7	
Not a trainee	69.6	19.4	11.0	
Tobacco use				0.27
No	67.8	20.0	12.2	
Yes	69.7	21.1	9.2	

Note: Referent group is emotional eating: never; numbers in bold indicate differences from the referent group significant at the $p < 0.05$ level.

In adjusted regression models of BMI or BC failure, those who reported EE behaviors "sometimes" or "often" had BMIs that were significantly higher and they had higher odds of failing BC standards than those who did not report emotional eating behaviors (Table 2).

The conditional process analysis models did not reveal gender as a statistically significant moderator in the relationship between PS, EE, and, BMI ($p = 0.83$) or BC failure ($p = 0.57$), therefore these results are not reported. In simple mediation models, PS indirectly influenced BMI and BC failure through its effect on emotional eating behaviors (Table 3), thus emotional eating appeared to mediate the relationship between PS and BMI or BC failure. Statistical tests did not show evidence that PS influenced BMI independently of its effect on EE behaviors. For BC failure, both the direct effect and indirect effect was statistically significant demonstrating that PS may affect BC failure directly and through EE as a mediator.

4. Discussion

This study examined the relationship between PS, BMI, BC failure, and gender among US Army Soldiers to determine if EE behaviors may mediate associations between PS and outcomes of BMI and BC failure.

Table 2
Results of regression models of body mass index or body composition failure by perceived stress and frequency of emotional eating among Soldiers.

	Body mass index N = 1,451[a]		Body composition failure N = 1,183[b]	
	b	*p*	OR	95% CI
Predictor/mediator				
Perceived stress score	0.03	0.095	1.04	[1.00, 1.08]
Emotional eating: sometimes	0.65	0.004	1.65	[1.11, 2.46]
Emotional eating: often	1.19	< 0.001	2.29	[1.43, 3.65]
Control variables				
Women	−2.09	< 0.001	1.48	[0.95, 2.30]
Recruit status: not a trainee	1.10	< 0.001	0.95	[0.48, 1.90]
Mean age	0.14	< 0.001	1.07	[1.03, 1.10]
Non-Hispanic Black	0.72	< 0.001	1.28	[0.82, 2.00]
Non-Hispanic other	0.01	0.960	0.94	[0.54, 1.62]
Hispanic	0.64	0.005	1.46	[0.97, 2.20]
Some college	0.37	0.061	0.83	[0.58, 1.20]
Degree	−0.11	0.727	0.40	[0.21, 0.77]
Married	0.54	0.006	1.35	[0.95, 1.93]
Use tobacco	0.25	0.209	1.27	[0.90, 1.80]
Physical activity (min/week)	0.19	0.025	0.99	[0.85, 1.15]
Constant	19.80	< 0.001	0.16	[0.005, 0.06]

Note: Referent group was no emotional eating, men, trainees, non-Hispanic White, high school education, single, no tobacco. Level of significance: $p < 0.05$.
[a] Model excluded 9 Soldiers with a missing BMI observation.
[b] Model excluded 277 Soldiers who had never been tested for body composition.

Table 3
Mediation effect of emotional eating on the association between perceived stress and outcomes of body mass index or body composition failure among Soldiers.

	Model		Model	
	Body Mass Index N = 1,333[a]		Body Comp. Failure N = 1,096[b]	
Paths	*b*	*p*	OR	95% CI
Perceived stress score to emotional eating	0.03	0.12	1.04	1.01, 1.08
Emotional eating to BMI or body comp. failure	0.72	< 0.001	1.53	1.21, 1.65
Effects	***b***	**95% CI**	**OR**	**95% CI**
Direct effect (*c'*)	0.03	-0.01, 0.07	1.04	1.01, 1.08
Indirect effect (*ab*)	0.03	0.02, 0.04	1.02	1.01, 1.03

Note: *b*, unstandardized regression coefficient; 95% CIs generated from 10,000 bootstrap samples. Models adjusted for training status (initial trainee or not a trainee), gender, age, race/ethnicity, level of education, marital status, tobacco use, and physical activity. Level of significance: $p < 0.05$.
[a] Model excluded 127 Soldiers with missing data.
[b] Model excluded 364 Soldiers (277 had not taken the BC test), 87 had missing data.

The results supported the mediational hypothesis, such that a positive relationship between PS and study outcomes was mediated by the frequency of EE behaviors. Although gender differences in the frequency of EE were observed, gender differences did not moderate the relationship between EE and BMI or BC failure.

The mean PS score of Soldiers in this sample was in the moderate range which is likely attributable to the multiple, unique sources of stress faced by many Soldiers. Military service routinely ranks as one of the most stressful professions (https://www.careercast.com/jobs-rated/-most-stressful-jobs, 2018), nonetheless, stress is an understudied, but important health-modifying factor among military populations. The results of this study support this assertion, demonstrating a relationship between PS, and BMI and BC failure, which are critically related to military service requirements (https://armypubs.army.mil/epubs/DR_pubs/DR_a/pdf/web/ARN7779_AR600-9_FINAL.pdf, 2019).

The association between PS and BMI and BC failure was mediated by EE. This finding emphasizes the potential importance of addressing EE given that Soldiers must maintain a body composition that is compliant with Army standards for career success. In this study, Soldiers who reported EE behaviors "sometimes," were nearly two times more likely to fail body composition standards and were 3.25 pounds heavier (0.65 BMI points) than those who did not report EE behaviors. Those who reported EE behaviors "often," were two times more likely to fail body composition standards and weighed 6.0 pounds heavier (1.19 BMI points) than those who did not report EE behaviors. The Army's BMI-based screening tables allow for variation in weight, but with an estimated 50% of Soldiers currently exceeding maximum allowed weight (https://phc.amedd.army.mil/topics/campaigns/hof/Pages/default.aspx, 2017), and the link between obesity and elevated injury risk (Knapik et al., 2007), the need to address behaviors that affect Soldiers' body weight status is increasingly critical.

These findings add to a growing body of literature examining modifiable behaviors affecting body weight and composition in military populations. Recent studies on the effects of intuitive eating characteristics (Cole, Clark, Heileson, DeMay, & Smith, 2016) or affiliation with a healthy eating identity (Jayne et al., 2018) have shown that understanding and leveraging individual motivations to eat may help the Army meet goals for reducing obesity among Soldiers. Like health promotion messaging aimed at decreasing obesity nationally (Roberto, Swinburn, Hawkes, et al., 2015), the Army uses campaign such as the Total Force Fitness Framework which encompasses a variety of programs aimed at improving nutrition, among other health behaviors (https://www.hprc-online.org/page/total-force-fitness, 2018). Prospective research is needed to examine the ways that food choice behaviors, such as EE, developing a healthy eating identity, and mindful eating may be related to health outcomes of importance to the Army. With the emphasis on increased readiness and improved physical performance among the force (Lacdan, 2017), further research on behavioral mediators that may influence dietary intake could support obesity reduction and performance objectives.

The results of this study are consistent with research showing EE behaviors to be related to weight status in a variety of populations (Ozier et al., 2008; Solomon, 2001; Torres & Nowson, 2007). No studies have examined this association in military personnel, and few have done so in other like service professionals who have high chronic stress (Cole et al., 2016). Studies on this topic have focused on the effects of PTSD or traumatic experiences on weight status (Dorflinger & Masheb, 2018; Jacobson, Smith, Smith, et al., 2009; Mattocks et al., 2012), rather than on the influence of perceived stress on eating behaviors. This study is consistent with literature showing women to be more likely to engage in EE behaviors (Cole et al., 2016; Torres & Nowson, 2007). Male and female Soldiers reported similar PS levels, but female Soldiers reported more EE behaviors, suggesting that, like civilian populations (Hawks, Merrill, Gast, & Hawks, 2004), there are gender differences in how Soldiers cope with stress and that some military women's eating behaviors may be negatively influenced by their military experience (Breland, Donalson, Nevedal, Dinh, & Maguen, 2017). While interactions between EE and gender were not found, over 80% of study participants were men, and, consequently, this study may have been underpowered to detect gender differences in the effect of EE on study outcomes. There remains a need to determine whether gender differences exist in how Soldiers and other public service professionals for whom stress is an unavoidable occupational hazard cope with stress.

Several studies have documented the need for nutrition to be improved among Soldiers (Purvis, Lentino, Jackson, Murphy, & Deuster, 2013; Smith, Dotson, Young, et al., 2013), but few studies have

identified specific factors underlying food choice. This study did not examine the longitudinal effects of PS on body weight status, but it is plausible that habitual EE increases the risk of higher BMI and failing BC standards over time. A limitation of this study was that BC failure was self-reported, that the timing of BC failure was not known, and that EE was measured by a two-item scale that has not been validated as a measure of EE. It is also acknowledged that some individuals respond to stress by reducing energy intake, and that the effects of stress in body weight status may be different in those individuals. For example, in this study the EE variable used in preliminary models included a third question that asked how frequently participants ate *less* when stressed. When that question was included in the EE variable, models showed a smaller effect size for the association between PS and body weight status, but the association remained significant, indicating that PS may have a powerful effect on body weight status, especially among those for which EE is a coping method. Additionally, the study population was of primarily young, healthy adults and results may not be generalizable to other groups.

5. Conclusion

This study found relationships between PS, EE, and body weight status among Soldiers. The results emphasize the need for stress to be considered a harmful influence on force readiness, and identified EE as a behavior that may mediate the relationship between stress and body weight. Since food choice behaviors are often modifiable, emotional eating behaviors should be researched further to better understand if there is a causal relationship with health outcomes and to investigate how reduction of less healthy food choice behaviors can be targeted by health promotion interventions aiming to reduce obesity in both military and civilian populations.

CRediT authorship contribution statement

Julianna M. Jayne: Conceptualization, Formal analysis, Methodology, Writing - original draft, Writing - review & editing. **Rachel Ayala:** Conceptualization, Writing - original draft, Writing - review & editing. **J. Philip Karl:** Conceptualization, Funding acquisition, Writing - review & editing. **Bethany A. Deschamps:** Conceptualization, Writing - review & editing. **Susan M. McGraw:** Data curation, Writing - review & editing. **Kristie O'Connor:** Writing - review & editing. **Adam J. DiChiara:** Writing - review & editing. **Renee E. Cole** Conceptualization, Funding acquisition, Methodology, Writing - review & editing.

Acknowledgements

The Soldiers who participated in this study are gratefully acknowledged along with Dr. Andrew Young for providing editorial assistance.

Role of funding source

Funding for this study was provided by the US Army Medical Research and Development Command (MRDC). The MRDC did not influence the study design, collection, analysis or interpretation of the data, writing the manuscript, or the decision to submit the paper for publication.

Disclaimer

The views expressed in this article are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the US Government.

Declaration of competing interest

The authors declare there is no conflict of interest.

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ISSN 2472-1972

Effects of Testosterone Supplementation on Ghrelin and Appetite During and After Severe Energy Deficit in Healthy Men

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Background: Severe energy deficits cause interrelated reductions in testosterone and fat free mass. Testosterone supplementation may mitigate those decrements, but could also reduce circulating concentrations of the orexigenic hormone ghrelin, thereby exacerbating energy deficit by suppressing appetite.

Objective: To determine whether testosterone supplementation during severe energy deficit influences fasting and postprandial ghrelin concentrations and appetite.

Design and methods: Secondary analysis of a randomized, double-blind trial that determined the effects of testosterone supplementation on body composition changes during and following severe energy deficit in nonobese, eugonadal men. Phase 1 (PRE-ED): 14-day run-in; phase 2: 28 days, 55% energy deficit with 200 mg testosterone enanthate weekly (TEST; n = 24) or placebo (PLA; n = 26); phase 3: free-living until body mass recovered (end-of-study; EOS). Fasting and postprandial acyl ghrelin and des-acyl ghrelin concentrations and appetite were secondary outcomes measured during the final week of each phase.

Results: Fasting acyl ghrelin concentrations, and postprandial acyl and des-acyl ghrelin concentrations increased in PLA during energy deficit then returned to PRE-ED values by EOS, but did not change in TEST (phase-by-group, $P < 0.05$). Correlations between changes in free testosterone and changes in fasting acyl ghrelin concentrations during energy deficit ($\rho = -0.42$, $P = 0.003$) and body mass recovery ($\rho = -0.38$; $P = 0.01$) were not mediated by changes in body mass or body composition. Transient increases in appetite during energy deficit were not affected by testosterone treatment.

Conclusions: Testosterone supplementation during short-term, severe energy deficit in healthy men prevents deficit-induced increases in circulating ghrelin without blunting concomitant increases in appetite.

Clinical Trials Registration: www.clinicaltrials.gov NCT02734238 (registered 12 April 2016).

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Keywords: hypothalamic-pituitary-gonadal axis, hypogonadism, androgen, military, weight cycling

Abbreviations: AUC, area under the curve; CI, confidence interval; ED, energy deficit; EOS, end-of-study time point; FM, fat mass; FT, free testosterone; LBM, lean body mass; PLA, placebo group; POST-ED, posttreatment time point; PRE-ED, pretreatment time point; TBM, total body mass; TEST, testosterone treatment group; TT, total testosterone

Received 13 December 2019
Accepted 26 February 2020
First Published Online 3 March 2020
Corrected and Typeset 27 March 2020

Severe energy deficits (~50%-100% of total daily energy expenditure) are common in military personnel during training [1, 2]. These deficits result from an inability or unwillingness to increase energy intake to match energy expenditures ranging from 4000 to 7000 kcal/d [3–5]. Consequences include total body mass (TBM) and fat free mass losses [6–10], and decreased physical performance [1]. Substantial reductions in circulating testosterone likely contribute to those decrements [6, 11–14]. Although body mass is regained and testosterone concentrations are restored by refeeding [6, 15], the amount of body mass regained has been reported to exceed the amount of TBM lost and be disproportionately comprised of fat mass (FM) [6, 16–18]. This "weight and fat overshoot" is thought to be caused, in part, by a physiologic drive to fully recover body protein stores [18–20], which may suggest reduced testosterone is a contributing factor.

Energy deficits also elicit adaptive responses in peripheral and central regulators of energy balance that stimulate appetite and promote TBM regain [21, 22]. One potential endocrine mediator of this response is ghrelin [23], a peptide hormone secreted primarily from the gastric mucosa in an acylated form that is rapidly converted into an unacylated (des-acyl) form that composes the majority of ghrelin in circulation [24, 25]. Acyl ghrelin exerts multiple physiologic effects [26], which include modulating glucose homeostasis and stimulating appetite and adipogenesis [27–31]. Des-acyl ghrelin also appears to regulate these outcomes, although effects may either reinforce or antagonize those of acyl ghrelin depending on the experimental context [32]. Nonetheless, circulating total (acyl + des-acyl) ghrelin increases in response to energy deficit [23, 33], and may contribute to a drive to eat that promotes regain of TBM and FM [21].

Circulating ghrelin concentrations may also be regulated by testosterone. Ghrelin is expressed in human testes [34, 35], and testosterone supplementation suppresses circulating total ghrelin in prepubertal boys and weight-stable, nonobese, eugonadal men [36, 37]. This effect, if present in nonobese men during severe energy deficit, may have the unintended and undesirable consequence of blunting energy deficit-induced increases in ghrelin and, subsequently, appetite. This could, in turn, exacerbate energy deficit. Recovery of TBM and FM during refeeding might also be slowed. Although effects of testosterone and other androgens on appetite regulation are poorly characterized [38], those responses would be consistent with studies reporting that testosterone supplementation is associated with gradual reductions in FM [39], and in some cases TBM [40]. However, testosterone supplementation has also been reported to increase circulating total ghrelin during energy balance [41] and have no effect during energy deficit [42] in men with overweight or obesity and low testosterone. The effect of testosterone supplementation on circulating ghrelin therefore remains uncertain and may depend on population demographics and/or be influenced by energy balance. Whether testosterone supplementation affects acyl and des-acyl ghrelin concentrations differently, and if any effects influence appetite and eating behavior has not been examined.

Our group recently published the results of a study designed to determine the effects of testosterone supplementation on changes in body mass and body composition during and following 28 days of severe energy deficit in healthy, nonobese, eugonadal young men [43]. Study results demonstrated that supplemental testosterone increased lean body mass during energy deficit. Herein, we report secondary outcomes included in the design of that study that addressed tertiary study objectives. Those objectives were to determine the effects of testosterone supplementation on circulating acyl and des-acyl ghrelin concentrations and appetite during and after severe energy deficit, and to explore relationships between changes in testosterone, ghrelin, appetite, TBM, and body composition during severe energy deficit and TBM recovery [44].

1. Materials and Methods

This study addressed tertiary objectives included in the Optimizing Performance for Soldiers study [43–45]. All outcomes presented herein were defined in the original study protocol.

The study was approved by the Pennington Biomedical Research Center (PBRC) institutional review board and the Human Research Protection Office of the US Army Medical Research and Materiel Command. Participants provided written informed consent, and the study was registered at www.clinicaltrials.gov as NCT02734238.

A. Subjects

Healthy, physically active men aged 18–39 years (mean ± SD = 25 ± 1 year), with total testosterone concentrations within normal physiological range (300 – 1000 ng/dL), and who met age-specific US Army body composition standards were recruited. Full inclusion and exclusion criteria and details of the recruitment process are published elsewhere [44].

B. Study Design

The study used a randomized, double-blind, placebo-controlled design. Full details of the Optimizing Performance for Soldiers study are published elsewhere [44], and only described in brief here.

The study consisted of a screening period followed by 3 separate study phases. During screening, volunteers recorded dietary intake for 3 consecutive days to assess usual energy intake. During phase 1 (study days 1–14), participants were free-living, but were provided a TBM-maintaining diet [44]. Immediately after completing phase 1, participants were admitted to the PBRC inpatient unit where they resided for 28 days during phase 2 of the study (study days 15–42). Participants were randomly assigned to one of 2 treatment groups for phase 2, testosterone (TEST), or placebo (PLA) [44]. Participants randomized to PLA received IM injections of 1 mL sesame oil on study days 15, 21, 28, and 35. Participants randomized to TEST received IM injections of 200 mg testosterone enanthate on the same days [44]. The only person not blinded to treatment allocation was the study pharmacist who prepared the treatments but did not interact with participants. During phase 2, dietary intake and physical activity were strictly monitored and controlled as described elsewhere [44], and prescribed to elicit a 55% energy deficit. Percent energy deficit did not differ between PLA (53 ± 4%) and TEST (52 ± 5%) [43].

Phase 3 (study day 43 to end-of-study [EOS]) began immediately upon completion of phase 2. Participants were released from the inpatient unit following testing on day 43, and instructed to return to their prestudy diet and exercise habits. All participants were followed for a minimum of 14 days (i.e., to study day 56). If, by day 56, a participant had returned to ±2.5% of his or her TBM measured on day 14 (as determined by average TBM over 3 consecutive days), EOS measurements were initiated and conducted over consecutive days (EOS, EOS + 1 day, EOS + 2 days). Otherwise, participants were followed until meeting the ±2.5% threshold, or until study day 84, whichever occurred first. These participants visited the laboratory weekly from day 57 to EOS for TBM measurement in addition to conducting daily TBM measurements at home. Therefore, the duration of phase 3 varied by participant and ranged from 14 to 42 days. Dietary intake during phase 3 was measured over 3 days the week before day 56 using the same methods used during screening. Study outcomes were measured during the last week of phase 1 (PRE-ED) and phase 2 (POST-ED), and at EOS as described below.

C. Appetite and Ghrelin Testing (Study Days 7, 43, and EOS + 1)

During the final week of each phase, on study days 7, 43, and EOS + 1, an experiment was conducted to determine the effects of testosterone supplementation on postprandial ghrelin and appetite responses during and after severe energy deficit (Fig. 1) [44]. Testing began at 07:00 hours on days 7 and 43, and at 07:00 hours (n = 27), 08:00 hours (n = 17), or 09:00 hours (n = 6) on EOS + 1. On each testing day, fasted participants consumed a standardized breakfast that provided energy equivalent to 20% of their individual total daily energy

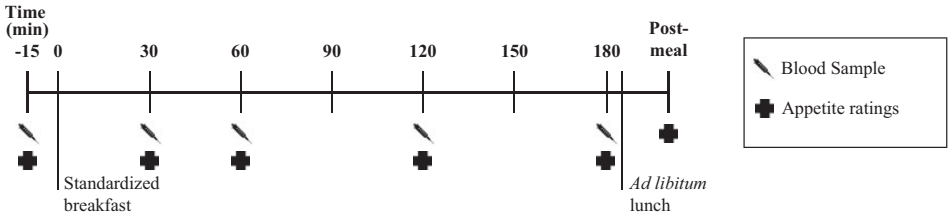

Figure 1. Study design. Appetite and ghrelin measurements were included as secondary outcomes in the Optimizing Performance for Soldiers study [43, 44]. On study days 7, 43, and EOS + 1, appetite ratings and fasted blood samples were collected 15 minutes before a standardized breakfast, and every 30–60 minutes after starting breakfast for 180 minutes. Appetite was also measured by energy intake during a lunch meal consumed ad libitum (185 minutes). Blood samples were collected to measure fasting insulin, fasting leptin, and fasting and postprandial glucose, acyl ghrelin, and des-acyl ghrelin. EOS, end of study.

expenditure determined during phase 1 (PLA: 515 ± 61 kcal, TEST: 542 ± 75 kcal; $P = 0.18$; percent energy from carbohydrate/fat/protein = 60/25/14). After 185 minutes, a lunch meal was served (percent energy from carbohydrate/fat/protein = 41/36/23) in a portion equivalent to ≥75% of each participant's total daily energy expenditure determined during phase 1. Participants were instructed to eat ad libitum until comfortably full, and the amount consumed was recorded. Blood samples were collected via intravenous catheter, and appetite was measured using visual analog scales 15 minutes before breakfast (served at time 0 minutes), and again 30, 60, 120, and 180 minutes after starting breakfast (Fig. 1). Appetite was measured again after lunch was finished. Visual analog scales were 100 mm in length and asked participants to rate their level of fullness, hunger, desire to eat, and the amount of food they thought they could eat (i.e., prospective consumption) at that moment [46]. Insulin, glucose, leptin, acyl ghrelin, and des-acyl ghrelin were measured at the -15 minute time point (i.e., fasting). Glucose, acyl-ghrelin, and des-acyl ghrelin concentrations were also measured at the 30, 60, 120, and 180 minute time points.

D. Biochemistries

Fasted blood samples were drawn on study days 0, 14, 42, and EOS between 06:00 hours and 09:00 hours (all day 42 measurements at 06:00 hours). Total testosterone (TT) was measured by automated immunoassay (Siemens Immulite 2000, Llanberis, UK) [47], and free testosterone (FT) was calculated according to the method of Vermeulen et al [48].

Blood samples were also drawn on appetite testing days (study days 7, 43, and EOS + 1) for measurement of fasting insulin and leptin, and fasting and postprandial glucose, acyl-ghrelin, and des-acyl ghrelin concentrations. Insulin was measured by automated immunoassay (Siemens Immulite 2000) [49]. Leptin was measured by radioimmunoassay (EMD Millipore Corporation, St Louis, MO) [50]. Glucose was measured using the Beckman DXC 600 Pro (Brea, CA). Acyl ghrelin was measured by radioimmunoassay (EMD Millipore Corporation) [51], and des-acyl ghrelin by ELISA (SPI Bio, Montigny le Bretonneux, France) [52]. All blood samples collected for measurement of acyl ghrelin were collected into chilled tubes containing 1 mg/mL 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride and were kept on ice until processing. After centrifugation, plasma acyl ghrelin aliquots were acidified using HCl to a final concentration of 0.05N. Des-acyl ghrelin was collected into chilled EDTA tubes and kept on ice until centrifugation. Samples were stored at -80°C until analysis. Total ghrelin concentration was calculated as the sum of acyl and des-acyl ghrelin.

E. Anthropometrics and Body Composition

Semi-nude, fasted, morning TBM was measured during laboratory visits using a calibrated digital scale (GSE Inc. Model 450, GSE Scale Systems, Novi, MI). Participants weighed themselves daily during phase 3 when they did not visit the laboratory using a calibrated

scaled provided by research staff (Body Trace, Inc. Model BT003, New York, NY). TBM, FM, and bone mass were determined by dual-energy X-ray absorptiometry (Lunar iDXA, GE Healthcare, Madison, WI) on study days 0, 11, 39, and EOS after an overnight fast. Lean body mass (LBM) was defined as TBM minus FM and bone mass.

F. Statistical Analysis

As described elsewhere [43, 44], sample size estimates were based on the primary study outcome, LBM, and indicated that 22 participants/group would provide 90% power to detect an expected 25% attenuation in LBM loss with testosterone supplementation. Sample size estimates for postprandial ghrelin area under the curve (AUC) were based on an expected 25% between-group difference following testosterone supplementation [36]. Using means and standard deviations from previous work by our laboratory conducted in a similar population [53], we calculated that an effect of that magnitude could be detected with 19 participants/group with 80% power and $\alpha = 0.05$.

All data were examined quantitatively and graphically, and logarithmic transformations were applied when necessary to meet model assumptions. Between-group comparisons of preintervention measurements were conducted using Student *t*-test or the Mann-Whitney *U*-test as appropriate. Linear mixed models with participant included as a random factor were used to examine effects of treatment, study day, and their interaction on fasting appetite ratings, glucose and hormone concentrations, body composition, and ad libitum energy intake at lunch. Models for testosterone and body composition were adjusted for day 0 values of each variable. The AUC with respect to ground ($y = 0$) was calculated for postprandial appetite ratings, and postprandial glucose and ghrelin concentrations using the trapezoidal method [54]. AUC was analyzed using linear mixed models as described above. Significant main effects and interactions were investigated further using between-group and within-group pairwise comparisons. Bonferroni corrections were applied to adjust for multiple comparisons. Because of evidence for between group differences in PRE-ED ghrelin concentrations (see Results), mixed models with PRE-ED values included as a covariate were also used to analyze between-group differences in fasting ghrelin concentrations and postprandial ghrelin AUC. Finally, in exploratory analyses, correlations between study outcomes were assessed using Pearson or Spearman rank correlations as appropriate, and multiple linear regression was used to assess relationships between variables while controlling for covariates.

Statistical analyses were completed using SPSS v24.0 (IBM, Armonk, NY). Tests were 2-sided and considered statistically significant at $P \leq 0.05$. Results are presented as mean ± SD or adjusted least square mean difference (95% confidence interval [CI]) unless otherwise noted.

2. Results

Of the 53 men who enrolled, 50 were randomized (60% non-Hispanic white, 26% non-Hispanic black, 6% Hispanic, 8% other) and all 50 completed the study [43]. Changes in testosterone concentrations and body composition measured between study days 0 to 56 have been previously reported [43]. To provide context for this secondary analysis, these changes are presented in Table 1 and briefly summarized here. EOS values have not been previously reported.

Within PLA, FT and TT concentrations were stable or declined from PRE-ED to POST-ED, then returned to PRE-ED concentrations by EOS; whereas, within TEST, FT and TT concentrations increased from PRE-ED to POST-ED, then decreased to below PRE-ED concentrations by EOS. Relative to PLA, concentrations of both TT and FT were higher in TEST at POST-ED but lower at EOS (Table 1). Changes in FM did not differ between groups (Table 1). In contrast, TBM loss from PRE-ED to POST-ED was attenuated in TEST relative to PLA because of an increase in LBM in TEST (Table 1). Participants

Table 1. Body Composition and Fasting Testosterone Concentrations

	PLA (n = 26)			TEST (n = 24)			P Value		
	PRE-ED (Day 11 or 14)	POST-ED (Day 39 or 42)	EOS	PRE-ED (Day 11 or 14)	POST-ED (Day 39 or 42)	EOS	Phase	Group	P × G
Body mass (kg)[1]	76.3 ± 10.2	71.3 ± 9.8[#]	74.9 ± 9.0[#‡]	80.2 ± 13.5	78.0 ± 12.3[#*]	81.0 ± 12.6[‡*]	<0.001	<0.001	<0.001
Fat mass (kg)[1§]	17.0 ± 6.7	12.3 ± 6.6	14.0 ± 5.4	16.6 ± 6.9	11.8 ± 6.1	13.7 ± 5.3	<0.001	0.67	0.93
Lean mass (kg)[1]	56.1 ± 5.1	55.8 ± 5.0	57.7 ± 5.1[#‡]	60.3 ± 10.1	62.8 ± 9.5[#*]	64.0 ± 10.2[#‡*]	<0.001	<0.001	<0.001
Total testosterone (ng/dL)[2]	402 ± 101	319 ± 131	424 ± 120[‡]	472 ± 153	1016 ± 246[#*]	324 ± 135[#‡*]	<0.001	<0.001	<0.001
Free testosterone (ng/dL)[2,3]	8.8 ± 2.3	5.0 ± 2.5[#]	8.8 ± 2.7[‡]	9.7 ± 2.4	24.4 ± 8.0[#*]	6.4 ± 2.7[#‡*]	<0.001	<0.001	<0.001

ED, energy deficit; EOS, end of study (return to initial body mass); PLA, placebo treatment group; TEST, testosterone treatment group.
Values are mean ± SD. Analyzed by linear mixed models with Bonferroni corrections, and adjusted using day 0 values.
*Significant difference from PLA at same time point ($P < 0.05$).
[#]Significant difference from PRE-ED within the same treatment group ($P < 0.05$).
[‡]Significant difference from POST-ED within the same treatment group ($P < 0.05$).
[§]Main effect of phase, all phases are significantly different ($P < 0.05$).
[1]Measured on study days 11 (phase 1), 39 (phase 2) and EOS.
[2]Measured on study days 14 (phase 1), 42 (phase 2) and EOS.
[3]Log_{10}-transformed for analysis.

in TEST (median [interquartile range] = 2 days [3]) then returned to their initial TBM earlier than those in PLA (11 days [16], $P = 0.001$). Five participants in PLA and 1 in TEST (Pearson $\chi^2 = 2.7$, $P = 0.10$) did not return to initial TBM within 42 days of ad libitum eating, which was the predetermined EOS for participants who did not fully recover lost TBM. As a result, EOS TBM in PLA remained 1.4 kg (95% CI: 0.4-2.5) lower than initial TBM ($P = 0.005$), but did not differ between the same time point in TEST (0.8 kg [95% CI: -0.3 to 1.9] $P = 0.22$).

A. Glucose and Hormone Concentrations

Testosterone supplementation had no effect on fasting leptin and insulin concentrations which were both lower at POST-ED relative to other time points (Table 2). Fasting glucose concentrations decreased in both groups from PRE-ED to POST-ED and were higher at EOS relative to PRE-ED in TEST but not PLA (Table 2). Testosterone supplementation had no effect on postprandial glucose AUC (data not shown).

Fasting acyl ghrelin concentrations were not statistically different between groups before treatment (i.e., at PRE-ED; $P = 0.16$). However, statistical adjustment for PRE-ED acyl ghrelin affected model results. When PRE-ED concentrations were not included in the model, a phase-by-group interaction was observed ($P = 0.05$). Fasting acyl ghrelin was found to increase from PRE-ED to POST-ED and then return to PRE-ED values at EOS in PLA but did not change over time in TEST (Table 2). In contrast, after statistically adjusting for PRE-ED concentrations, no significant treatment effects were observed (phase-by-group interaction, $P = 0.11$).

Postprandial acyl ghrelin AUC was higher in TEST relative to PLA before treatment ($P = 0.05$; Fig. 2C). However, statistical adjustment for PRE-ED acyl ghrelin AUC did not affect model results. When PRE-ED AUC was not included in the model, a phase-by-group interaction was observed ($P = 0.004$). Acyl ghrelin AUC increased from PRE-ED to POST-ED and then returned to PRE-ED values at EOS in PLA, but did not change over time in TEST (Fig. 2A-C). The phase-by-time interaction remained significant after adjusting for PRE-ED acyl ghrelin AUC (phase-by-group interaction, $P = 0.02$). Following the adjustment, POST-ED acyl ghrelin AUC was 21% higher in PLA relative to TEST (adjusted mean difference = 4189 pg × min/mL [95% CI: 1679-6699] $P = 0.001$), and no between group difference was observed at EOS ($P = 0.75$). Finally, when postprandial AUC was recalculated as a change from fasting concentrations, no effect of testosterone was observed (data not shown; phase-by-group interaction, $P = 0.58$), indicating that the effect of testosterone on acyl ghrelin AUC was primarily attributable to a change in fasting concentrations rather than a change in the magnitude of the postprandial response.

Fasting des-acyl ghrelin concentrations trended toward being statistically different between groups before treatment ($P = 0.06$). In the full cohort, fasting des-acyl ghrelin concentrations increased from PRE-ED to POST-ED and then decreased and did not differ from PRE-ED concentrations at EOS (Table 2). No effect of testosterone supplementation was observed whether or not PRE-ED des-acyl ghrelin was included in the model.

Postprandial des-acyl ghrelin AUC was higher in TEST relative to PLA before treatment ($P = 0.01$). However, statistical adjustment for PRE-ED des-acyl ghrelin AUC did not affect model results. When PRE-ED AUC was not included in the model, a phase-by-group interaction was observed ($P = 0.02$). Des-acyl ghrelin AUC increased from PRE-ED to POST-ED and then returned to PRE-ED values at EOS in PLA but did not change over time in TEST (Fig. 2D-F). The phase-by-time interaction remained significant after adjusting for the PRE-ED differences in des-acyl ghrelin AUC (phase-by-group interaction, $P = 0.05$). The ratio of the geometric mean of POST-ED des-acyl ghrelin AUC in PLA relative to that in TEST was 1.15 (95% CI: 1.03, 1.29; $P = 0.01$), and no between-group difference was observed at EOS ($P = 0.98$; phase-by-group interaction, $P = 0.02$). When postprandial AUC was recalculated as a change from fasting des-acyl ghrelin concentrations, no effect of testosterone was observed (data not shown; phase-by-group interaction, $P = 0.97$), suggesting

Table 2. Fasting Glucose, Insulin, Leptin and Ghrelin Concentrations, Fasting Appetite Ratings, and Energy Intake During an ad libitum Lunch Meal

	PLA (n = 26)			TEST (n = 24)			P Value		
	PRE-ED (Day 7)	POST-ED (Day 43)	EOS + 1 day	PRE-ED (Day 7)	POST-ED (Day 43)	EOS + 1 day	Phase	Group	P × G
Glucose (mg/dL)	86 ± 7	79 ± 6[#]	86 ± 7[‡]	85 ± 7	76 ± 6[#]	90 ± 6[#‡]	<0.001	0.98	0.01
Insulin (μIU/mL)[1†]	4.5 [6.3]	0.2 [2.7]	5.7 [6.4]	4.6 [3.8]	0.2 [2.1]	6.3 [5.9]	<0.001	0.56	0.97
Leptin (ng/mL)[1†]	6.2 [6.1]	3.2 [1.7]	5.8 [6]	5.4 [7.9]	3.2 [1.2]	4.8 [3.1]	<0.001	0.65	0.77
Acyl ghrelin (pg/mL)	118 ± 44	145 ± 59[#]	130 ± 49	137 ± 53	132 ± 59	141 ± 52	0.27	0.65	0.05
Des-acyl ghrelin (pg/mL)[1†]	163 [108]	204 [121]	199 [102]	228 [103]	244 [177]	228 [172]	<0.001	0.38	0.68
Total ghrelin (pg/mL)[1¶]	287 [168]	326 [233]	310 [160]	350 [180]	353 [273]	357 [276]	0.002	0.49	0.15
Acyl/des-acyl ghrelin ratio	0.65 ± 0.20	0.64 ± 0.25	0.71 ± 0.36	0.64 ± 0.30	0.49 ± 0.22	0.59 ± 0.21	0.08	0.49	0.15
Fullness (mm)[1†]	13 [33]	9 [23]	12 [34]	15 [39]	10 [32]	17 [34]	0.01	0.91	0.60
Hunger (mm)[¥]	59 ± 24	73 ± 16	68 ± 22	62 ± 21	75 ± 18	73 ± 15	<0.001	0.46	0.85
PC (mm)	66 ± 19	74 ± 20	70 ± 21	69 ± 19	75 ± 17	73 ± 19	0.12	0.75	0.81
DtE (mm)[†]	62 ± 26	76 ± 20	68 ± 23	66 ± 25	77 ± 19	70 ± 22	0.001	0.64	0.86
Energy intake at lunch meal (kcal)[†]	1211 ± 438	1418 ± 560	1108 ± 455	1145 ± 366	1581 ± 544	1241 ± 468	<0.001	0.52	0.11

DtE, Desire to eat; ED, energy deficit; EOS, end of study (return to initial body mass); PLA, placebo treatment group; PC, prospective consumption; TEST, testosterone treatment group.

Values are mean ± SD or median [IQR]. Analyzed by linear mixed models with Bonferroni corrections.

*Significant difference from PLA at same time point ($P < 0.05$).

[#]Significant difference from PRE-ED within the same treatment group ($P < 0.05$).

[‡]Significant difference from POST-ED within the same treatment group ($P < 0.05$).

[†]Main effect of phase, POST-ED differs from PRE-ED and EOS.

[¶]Main effect of phase, POST-ED differs from PRE-ED only.

[¥]Main effect of phase, PRE-ED differs from POST-ED and EOS.

[1]Log_{10}-transformed for analysis.

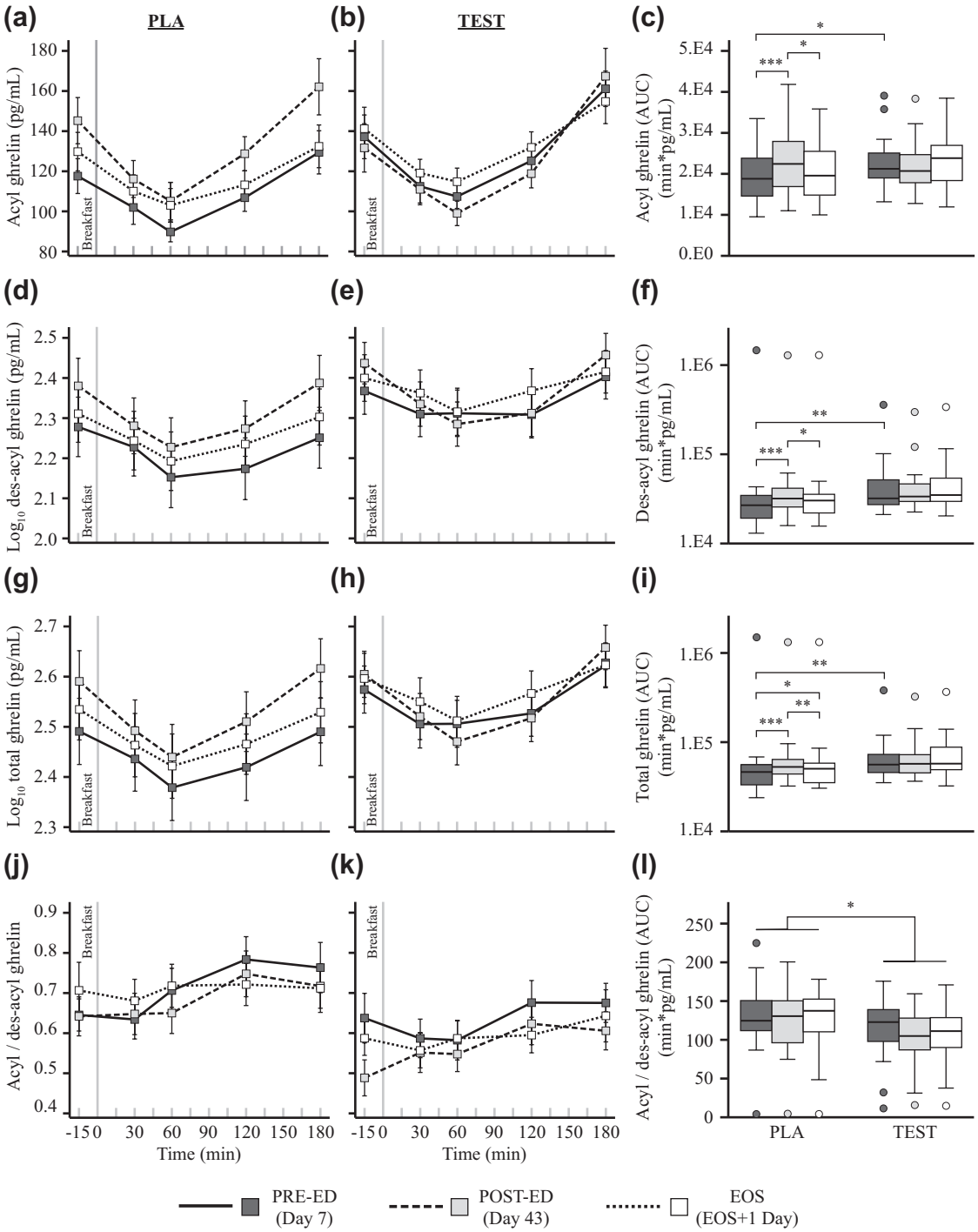

Figure 2. Testosterone supplementation alters postprandial ghrelin responses during energy deficit. Fasting and postprandial acyl ghrelin (A-C), des-acyl ghrelin (D-F), total ghrelin (G-I), and the acyl ghrelin/des-acyl ghrelin ratio (J-L) measured during a standardized meal challenge (0–180 minutes) administered before (PRE; study day 7) and after (POST; study day 43) severe energy deficit (ED), and 1 day after body mass recovery (EOS). (A-B, D-E, G-H, J-K) Individual data points are mean ± SEM. (C, F, I, L) Boxes span 25th to 75th percentile with the median value represented by a solid line inside the box. Whiskers extend to minimum and maximum values or to 1.5 times the interquartile range if any values outside that range (represented by circles). Main effects of group, study phase, and their interaction on area under the postprandial response curve (AUC) analyzed by linear mixed models with Bonferroni corrections. (C, F, I) Group-by-phase interaction, $P < 0.05$. (L) Main effect of group, $P = 0.05$. (C, F, I, L) * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. PLA, placebo treatment group; TEST, testosterone treatment group.

that the effect of testosterone on des-acyl ghrelin AUC was attributable to a combination of changes in fasting concentrations and in the magnitude of the postprandial response.

Results for fasting total ghrelin and total ghrelin AUC largely mirrored those for des-acyl ghrelin which was the predominant form in circulation (Table 2 **and** Fig. 2G-I). Testosterone supplementation had no effect on the ratio of acyl to des-acyl ghrelin under either fasting or postprandial conditions (Table 2 **and** Fig. 2J-L).

B. Appetite

Fasting and postprandial appetite ratings demonstrated an increase in hunger, desire to eat, prospective consumption, and a decrease in fullness from PRE-ED to POST-ED that was fully or partially resolved by EOS and not affected by testosterone supplementation (Table 2, Fig. 3). Changes in energy intake during the ad libitum lunch mirrored that response and did not differ by study group (Table 2). The difference in self-reported energy intake measured during the first week of phase 3 relative to that measured during screening also did not differ between groups (PLA: 347 kcal/d [95% CI: -171 to 864] vs. TEST: 900 kcal/d [95% CI: 432-1368], $P = 0.11$). However, the median [IQR] rate of TBM regain from POST-ED until a participant was considered returned to their initial TBM was higher in TEST (1.7 kg/d [1.9]) relative to PLA (0.4 kg/d [1.1]; $P = 0.03$).

C. Associations Between Changes in Testosterone, Ghrelin, and Appetite

In the full cohort, changes in TT and FT concentrations were inversely correlated with changes in fasting acyl ghrelin, but not fasting des-acyl ghrelin, concentrations (Table 3). The correlations between changes in FT concentrations and changes in fasting acyl ghrelin concentrations remained statistically significant after adjusting for changes in FM, LBM, and TBM (Table 4).

Changes in postprandial acyl ghrelin, des-acyl ghrelin, total ghrelin, and the acyl/des-acyl ghrelin ratio AUCs from PRE-ED to POST-ED were not correlated with changes in postprandial hunger AUC during the same period, or with changes in ad libitum energy intake as measured by: 1) the difference in self-reported energy intake during the first week of phase 3 relative to screening; 2) the difference in lunch meal ad libitum energy intake at POST-ED relative to PRE-ED; or 3) the rate of TBM regain from POST-ED until initial TBM was reached (data not shown).

3. Discussion

In this secondary analysis, testosterone supplementation during severe energy deficit prevented deficit-induced increases in fasting acyl ghrelin concentrations, and in postprandial acyl and des-acyl ghrelin concentrations in nonobese, healthy men. Despite an established role for ghrelin in energy balance regulation, these effects did not blunt energy deficit-induced increases in appetite, slow TBM recovery, or impact changes in FM. These findings confirm and extend evidence supporting an inhibitory effect of testosterone supplementation on circulating ghrelin in healthy men, but suggest this effect is not sufficient to suppress appetite during energy deficit or subsequent body mass recovery.

Whereas previous studies have examined associations between testosterone supplementation and fasting total ghrelin concentrations [36, 37, 41, 42], to our knowledge, this study is the first to examine relationships between testosterone and both fasting and postprandial acyl and des-acyl ghrelin forms. This is a strength of the study, and important to developing a complete understanding of any effects of testosterone supplementation on ghrelin because: 1) acyl ghrelin is considered the more biologically active ghrelin form [26]; 2) acyl and des-acyl ghrelin may have independent, and, in some cases, opposing effects [32]; and 3) most individuals spend the majority of the day in a postprandial state [55, 56]. Using this comprehensive approach, this study extends the findings of Gambineri et al [36], who demonstrated

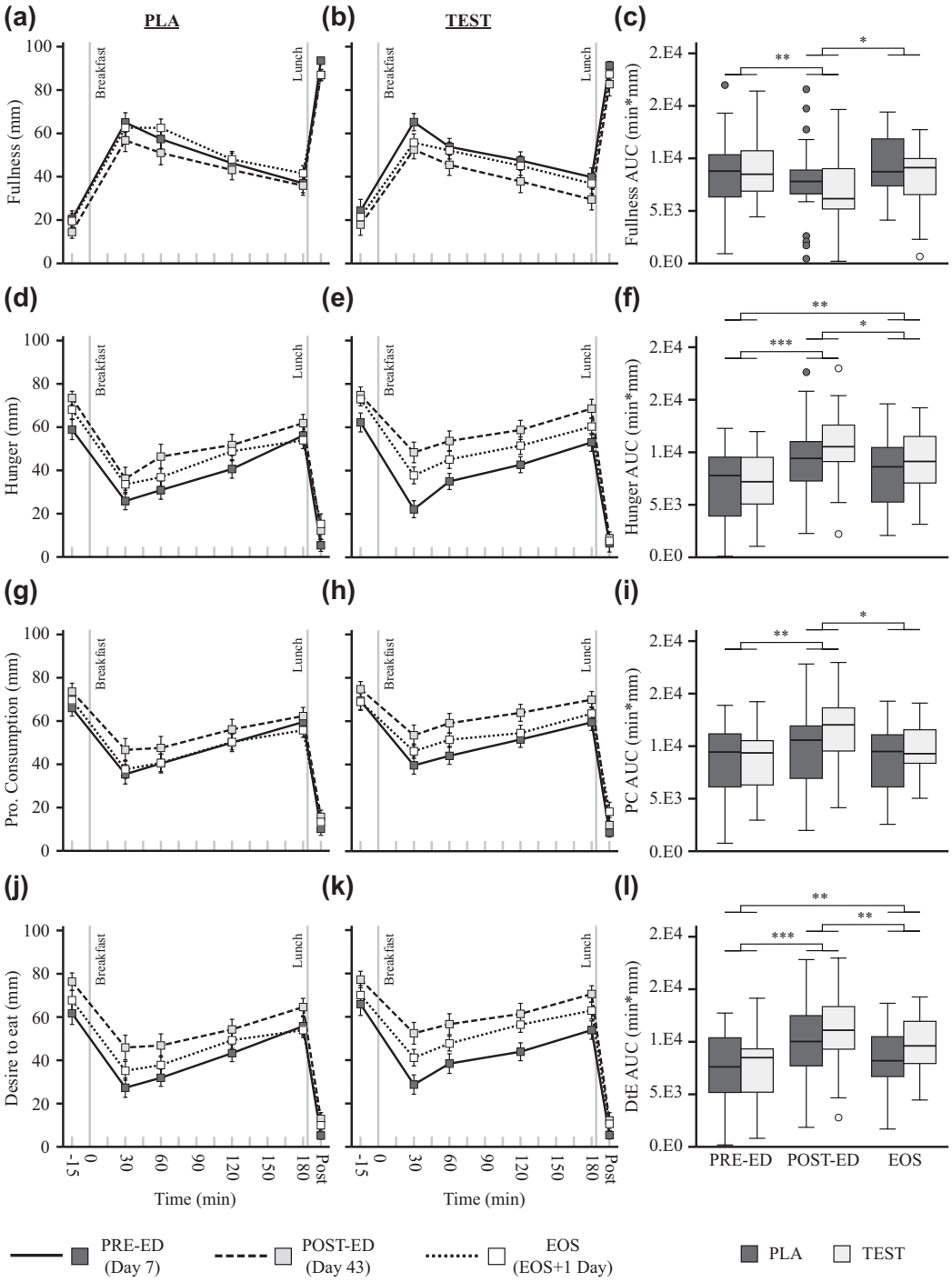

Figure 3. Testosterone supplementation does not affect appetite during energy deficit. Fasting and postprandial fullness (A-C), hunger (D-F), prospective consumption (Pro. Consumption, G-I), and desire to eat (J-L) measured during a standardized breakfast (0–180 minutes) followed by an ad libitum lunch meal (served at 185 minutes) administered before (PRE; study day 7) and after (POST; study day 43) severe energy deficit (ED), and one day after body mass recovery (EOS). (A-B, D-E, G-H, J-K) Individual data points are mean ± SEM. (C, F, I, L) Boxes span 25th to 75th percentile with the median value represented by a solid line inside the box. Whiskers extend to minimum and maximum values or to 1.5 times the interquartile range if any values outside that range (represented by circles). Main effects of group, study phase, and their interaction on area under the postprandial response curve (AUC) analyzed by linear mixed models with Bonferroni corrections. (C, F, I, L) Main effect of phase, $P < 0.05$. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$. PLA, placebo treatment group; TEST, testosterone treatment group.

Table 3. Correlations Between Changes in Fasting Ghrelin Concentrations and Changes in Testosterone and Body Mass Outcomes

	ΔdaG	ΔtotG	ΔaG/daG	ΔTT	ΔFT	ΔFM	ΔLBM	ΔTBM
ΔPRE-ED to POST-ED								
Δacyl ghrelin	0.46***	0.69***	0.40**	-0.37**	-0.42**	0.12	-0.12	-0.04
Δdes-acyl ghrelin		0.88***	-0.48***	-0.03	-0.04	0.16	-0.05	0.01
Δtotal ghrelin			-0.12	-0.23	-0.23	0.20	-0.11	-0.03
Δacyl/des-acyl ghrelin				-0.26	-0.34*	-0.03	-0.11	-0.13
ΔPOST-ED to EOS								
Δacyl ghrelin	0.41**	0.75***	0.45***	-0.39**	-0.38**	-0.42**	-0.15	-0.31*
Δdes-acyl ghrelin		0.86***	-0.45***	-0.14	-0.12	-0.36*	0.01	-0.18
Δtotal ghrelin			-0.04	-0.31*	-0.30*	-0.41**	-0.06	-0.27
Δacyl/des-acyl ghrelin				-0.17	-0.22	-0.09	-0.13	-0.15

n = 49–50. Δchange; aG, acyl ghrelin; daG; des-acyl ghrelin; FM, fat mass; FT, free testosterone; LBM, lean body mass; TBM, total body mass; TT, total testosterone. Values are Pearson's correlation coefficients or Spearman's rho. All correlations were computed using change scores calculated over the same time period for the independent and dependent variables (ie, PRE-ED to POST-ED and POST-ED to EOS).
*$P < 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

that supplementing testosterone to supraphysiologic concentrations (~1400ng/dL; ~60% increase from baseline) resulted in a ~25% decrease in fasting total ghrelin concentrations over 8wk in normal-weight men in whom endogenous testosterone production was suppressed by oral progestin administration. The present study demonstrates that those results may be driven more by changes in acyl ghrelin than des-acyl ghrelin, and that the effects on acyl ghrelin concentrations can be produced at testosterone concentrations that span the lower to upper limits of the normal physiologic range (300-1000 ng/dL [57]; 115% increase from baseline). Further, these findings demonstrate that testosterone supplementation does not appear to measurably impact the magnitude of the postprandial acyl or des-acyl ghrelin response independent of changes in fasting concentrations (Table 2 and Fig. 2).

Changes in TBM, FM, and LBM did not mediate associations between testosterone and fasting acyl ghrelin concentrations (Table 4). This suggests that the observed effects of testosterone on acyl ghrelin were independent of changes in TBM and body composition, both of which have previously been associated with ghrelin concentrations [26]. The result contrasts with a recent study in which decreased TBM and FM were hypothesized to override any effect of testosterone treatment on fasting total ghrelin concentrations in men with obesity and low-normal testosterone concentrations who were consuming a very low energy diet [42]. Whether the discrepancy is due to the absence of acyl ghrelin measurements in that study, or due to differences in the study populations, the magnitude of TBM and FM loss or the method used to induce energy deficit (increased energy expenditure vs. very low energy diet) is unclear. Additionally, it has been postulated that any effect of testosterone on ghrelin concentrations may be indirect and mediated through changes in insulin or leptin concentrations [58]. However, neither insulin nor leptin was affected by testosterone supplementation in this study. Of note, multiple endocrine, neural, and nutritive factors collectively regulate basal and periprandial ghrelin concentrations, and the combinations of those factors underpinning ghrelin responses to body mass loss are not fully characterized [26, 58]. Study findings strengthen evidence that testosterone may be 1 of those factors. Conversely, ghrelin has been shown to suppress hypothalamic-pituitary-gonadal axis activity [59, 60], and an energy deficit-induced increase in ghrelin is thought to be 1 mechanism by which reproductive function is suppressed when energy availability is low [61, 62]. Taken together, these observations add support for a bidirectional interaction between testosterone and ghrelin linking energy homeostasis and male reproductive function.

Despite preventing energy deficit-induced increases in acyl ghrelin, testosterone supplementation did not blunt deficit-induced increases in appetite, or slow recovery of TBM

Table 4. Association Between Changes in Fasting Acyl Ghrelin Concentrations and Changes in Free Testosterone (FT) Concentrations are not Mediated by Changes in Body Composition or Body Mass

	Model 1[a]		Model 2		Model 3		Model 4	
	β [95% CI]	P	β [95% CI]	P	β [95% CI]	P	β [95% CI]	P
ΔPRE-ED to POST-ED								
ΔFT (ng/dL)	-1.7 [-3.0, -0.4]	0.01	-1.7 [-3.1, -0.4]	0.01	-2.6 [-4.6, -0.6]	0.01	-2.7 [-4.6, -0.8]	0.01
Age (yr)	-2.5 [-5.6, 0.7]	0.12	-2.4 [-5.6, 0.7]	0.13	-2.3 [-5.5, 0.8]	0.14	-2.3 [-5.4, 0.9]	0.15
Baseline BF (%)	0.3 [-2.1, 2.7]	0.78	0.7 [-2.2, 3.7]	0.61	0.2 [-2.2, 2.6]	0.84	0.9 [-1.6, 3.5]	0.45
ΔFM (kg)	-		3.9 [-12.0, 20.0]	0.62	-		-	
ΔLBM (kg)	-		-		6.3 [-4.4, 1.7]	0.24	-	
ΔTBM (kg)	-		-		-		6.8 [-2.9, 16.5]	0.16
Constant	74.3 [-2.8, 151.5]	0.06	83.2 [-2.5, 168.9]	0.06	70.8 [-6.2, 147.9]	0.07	85.8 [7.7, 163.8]	0.03
R^2	0.20	0.02	0.20	0.04	0.22	0.02	0.23	0.02
ΔPOST-ED to EOS								
ΔFT (ng/dL)	-1.5 [-2.6, -0.3]	0.01	-1.4 [-2.5, -0.2]	0.02	-1.6 [-2.9, -0.4]	0.01	-1.5 [-2.7, -0.2]	0.02
Age (yr)	0.8 [-2.3, 3.9]	0.59	0.2 [-3.1, 3.5]	0.90	0.7 [-2.5, 3.9]	0.67	0.8 [-2.3, 4.0]	0.59
Baseline BF (%)	3.0 [0.6, 5.3]	0.01	2.0 [-0.9, 4.8]	0.16	3.1 [0.7, 5.6]	0.01	2.9 [0.3, 5.6]	0.03
ΔFM (kg)	-		9.7 [-2.6, 6.4]	0.23	-		-	
ΔLBM (kg)	-		-		2.5 [-6.4, 1.1]	0.57	-	
ΔTBM (kg)	-		-		-		-0.2 [-7.1, 6.7]	0.95
Constant	-97.1 [-173.3, -20.9]	0.01	-42.7 [-160.6, 75.3]	0.47	-101.5 [-179.9, -23.1]	0.01	-95.6 [-187.8, -3.3]	0.04
R^2	0.28	0.002	0.30	0.003	0.29	0.004	0.28	0.005

n = 49. Δ, change; BF, body fat; FM, fat mass; LBM, lean body mass; TBM, total body mass.

[a]All models are multiple linear regression analyses. Dependent variable is change in fasting acyl ghrelin concentration over the time period indicated in the table.

doi: 10.1210/jendso/bvaa024 | Journal of the Endocrine Society | 13

and FM during refeeding. These results are incongruent with acyl ghrelin's putative role as an orexigenic and adipogenic hormone. However, clinical studies demonstrating acute orexigenic effects of ghrelin have generally used IV infusions resulting in supraphysiologic acyl and total ghrelin concentrations [63]. Studies conducted in normal-weight adults using lower dose infusions that produce more physiologically relevant ghrelin concentrations have not reported statistically significant increases in appetite despite 1.6- to 2.4-fold increases in circulating acyl and total ghrelin [64, 65]. Further, some [66–68], but not all [30], rodent studies suggest that des-acyl ghrelin may antagonize the appetite-stimulating effects of acyl ghrelin. We therefore posit that the ~1.25-fold between-group difference in changes in fasting acyl-ghrelin concentrations in this study were likely not sufficient to result in between-group differences in hunger, energy intake or changes in FM in the absence of differences in the acyl:des-acyl ghrelin ratio. Additionally, eating behavior is influenced by myriad interactions among endocrine, neural, and other factors [69] that are sensitive to energy deficit, and promote an appetitive drive to regain lost body mass [22]. Responses of those factors to energy deficit may have overwhelmed any influence of testosterone-mediated differences in ghrelin on appetite.

Strengths of this study include the lack of attrition, strict control of energy intake and expenditure, assessment of postprandial acyl and des-acyl ghrelin dynamics, and concomitant measurements of appetite. However, results should be interpreted within the context of several limitations. First, as ghrelin and appetite were secondary outcomes, the trial was not designed specifically to examine interactions between those outcomes and testosterone. Second, despite randomization, between-group differences in acyl ghrelin and des-acyl ghrelin AUC were noted before testosterone treatment. However, when statistical adjustment for the pretreatment differences was used, both acyl ghrelin AUC and des-acyl ghrelin AUC were higher at the POST-ED time point in PLA relative to TEST. Further, in confirmatory analysis of covariance analyses adjusting for PRE-ED ghrelin AUC, changes in acyl and des-acyl ghrelin AUCs from PRE-ED to POST-ED remained greater in PLA relative to TEST (data not presented). Taken together, these results are consistent with a testosterone-mediated suppression of ghrelin. Because no statistically significant between-group differences in changes in fasting ghrelin concentrations were observed, these findings also highlight the value of obtaining serial measurements for appetite-associated hormones that deviate from fasting concentrations throughout much of the day.

An additional study limitation is that mean TT concentrations in PLA did not fall as precipitously as has been reported in studies of military personnel experiencing severe energy deficit, and the substantial LBM losses reported in those studies were not reproduced [6, 12]. The presence of additional physiologic stressors in previous studies, such as sleep deprivation, likely explain these differences. However, we previously reported that PLA did experience reductions in luteinizing hormone in addition to reductions in FT, and increases in sex-hormone binding globulin during energy deficit [43]. Those responses are consistent with hypothalamic-pituitary-gonadal axis suppression [57]. Therefore, study findings can to some extent be extrapolated to the real-life conditions the study was intended to mimic. Results may also be generalizable to physiologic adaptations experienced in men in whom energy imbalances result from intentional or unintentional restriction of energy intake and increases in energy expenditure during athletic training [61]. Finally, mean POST-ED testosterone concentrations in TEST were at the high end of the normal physiologic range for young men and substantially higher than PRE-ED concentrations. This allowed an examination of relationships between testosterone, ghrelin, and appetite across the full range of normal physiologic concentrations. However, whether similar effects would have been observed had testosterone concentrations been maintained closer to the normal basal range in this population cannot be determined.

4. Conclusions

Testosterone supplementation blunted energy deficit-induced increases in ghrelin, associations between changes in testosterone and ghrelin were not mediated by changes in

body composition, and effects of testosterone supplementation on ghrelin did not suppress appetite. Testosterone supplementation therefore appears unlikely to appreciably influence appetite or energy intake in nonobese young men, to include military personnel, during and following periods of unavoidable severe energy deficit. However, observations strengthen evidence of testosterone-ghrelin interactions, and provide additional insight into endocrine factors potentially linking energy homeostasis and male reproductive function.

Acknowledgments

We thank the study participants who participated in the study, the study team at the PBRC for significant contributions to study management and data collection, Dr. Shalender Bhasin for assistance in designing the testosterone intervention, and Philip Niro for assistance in designing the figures.

Financial Support: This work was funded by the US Department of Defense under the Collaborative Research to Optimize Warfighter Nutrition II and III projects and the Joint Program Committee-5. C.E.B. was supported in part by an appointment to the Department of Defense (DoD) Research Participation Program administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the U.S. Department of Energy (DoE) and the DoD. ORISE is managed by the Oak Ridge Associated Universities (ORAU) under DoE contract number DE-SC0014664.

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HIGH ALTITUDE MEDICINE & BIOLOGY
Volume 00, Number 00, 2020
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DOI: 10.1089/ham.2019.0109

High-Altitude Acclimatization Suppresses Hepcidin Expression During Severe Energy Deficit

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Abstract

Hennigar, Stephen R., Claire E. Berryman, Alyssa M. Kelley, Bradley J. Anderson, Andrew J. Young, James P. McClung, and Stefan M. Pasiakos. High-altitude acclimatization suppresses hepcidin expression during severe energy deficit. *High Alt Med Biol.* 00:000–000, 2020.

Background: The erythropoietic cells in the bone marrow require iron to synthesize heme for incorporation into hemoglobin. Exposure to hypoxic conditions, such as extended sojourns to high altitude (HA), results in increased erythropoiesis and an increased physiological requirement for iron. In addition to increasing iron requirements, hypoxic conditions suppress appetite and often lead to decreased energy intake. The objective of this study was to determine the combined effects of severe energy deficit and hypoxia on hepcidin and measures of iron status in lowlanders sojourning to HA.

Methods: Iron status indicators and hepcidin were determined in 17 healthy male volunteers (mean ± standard deviation, age 23 ± 6 years, body mass index 27 ± 4 kg/m^2) fed a controlled diet (12 ± 1.2 mg iron/day) during a 20-day sojourn to 4300 m above sea level.

Results: Chronic exposure to HA during severe energy deficit increased hematocrit by 12% ($p < 0.01$) and decreased serum hepcidin by 37% ($p < 0.01$) compared with baseline. Ferritin declined by 18% ($p = 0.02$) and transferrin saturation and soluble transferrin receptor increased by 55% and 83%, respectively ($p < 0.01$ for both) compared with baseline.

Conclusions: HA acclimatization suppresses hepcidin expression to increase iron availability during severe energy deficit. Registered at ClinicalTrials.gov as NCT02731066.

Keywords: erythropoiesis; hypoxia; iron

Introduction

Steady-state erythropoiesis in healthy human adults produces ~200 billion new erythrocytes each day, a process that requires 20–25 mg of iron daily (Muckenthaler et al., 2017). The physiological demand for iron increases by an estimated 8 mg iron per day to support the increased rate of erythropoiesis with exposure to hypoxic conditions, assuming the average increase in rate in red blood cell volume across all altitudes is ~50 mL per week (Sawka et al., 2000). The increased physiological demand for iron is thought to be facilitated, in part, by a suppression in the iron regulatory hormone hepcidin. Hepcidin is a 25-amino acid peptide hormone produced by the liver in response to iron loading, inflammation, and infection (Collins et al., 2008). Hepcidin signals for the internalization and degradation of ferroportin, the primary cellular iron export protein, thereby limiting iron efflux into circulation. Conversely, hepatic hepcidin mRNA is suppressed in mouse models of anemia and hypoxia (Nicolas et al., 2002).

Recent studies have observed significant declines in circulating concentrations of hepcidin after exposure to high altitude (HA) (3400–5400 m) (Piperno et al., 2011; Talbot et al., 2012). Declines in hepcidin are thought to facilitate iron availability for enhanced erythropoiesis with hypoxia. A major hurdle for Warfighters conducting dismounted operations or training in HA regions, particularly for extended periods, is maintaining energy balance. Sojourns to HA are

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also accompanied by a decrease in appetite (Butterfield, 1999), which is compounded by an increase in resting metabolic rate (Butterfield et al., 1992) and, oftentimes, increased physical activity. Prolonged energy deficit at HA results in greater weight loss with a higher percentage of total body mass loss coming from fat-free mass compared with that at sea level. Recently, we conducted a randomized controlled trial to determine the effects of dietary protein intake during energy deficit at HA on fat-free mass preservation (Berryman et al., 2017). We found that energy deficit during 21 days at 4300 m decreased fat-free mass regardless of protein intake. The objective of this secondary analysis was to determine the combined effects of severe energy deficit and hypoxia on hepcidin and measures of iron status in lowlanders during 20 days of controlled feeding at 4300 m above sea level.

Methods

The study conformed to the principles of the Declaration of Helsinki and was approved by the institutional review board at the U.S. Army Research Institute of Environmental Medicine (USARIEM) and registered at ClinicalTrials.gov as NCT02731066. Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to DoD Instruction 3216.02 and 32 CFR 219 on the use of human subjects in research.

Participants

Males, age 18–42 years, who were born at altitudes <2100 m and were physically active (programmed physical activity 2–4 day/week), were eligible to volunteer. Exclusion criteria included living or traveled to areas >1200 m for ≥5 days within the past 2 months, metabolic or cardiovascular abnormalities, anemia (hematocrit <38% and hemoglobin <12.5 g/dL), and blood donation within 8 weeks of beginning the study. Twenty-one participants were randomized and 17 participants completed the study and were included in the final analyses (Fig. 1). No participants displayed signs of excessive erythrocytosis during the study period.

Study design and procedures

Participants lived at sea level (Natick, MA; 50 m) for 21 days before traveling to HA. During this free-living and weight maintenance phase, participants followed dietary prescriptions administered by registered dietitians for energy and protein intake. On day 21, participants were flown to Denver, Colorado, and were provided supplemental oxygen to maintain normoxic oxygen saturation until they arrived at the USARIEM Maher Memorial Altitude Laboratory at the summit of Pikes Peak (4300 m) the next morning (peripheral oxygen saturation measured hourly from arrival to 2200 h and every 30 minutes beginning at 0300 h was 97.0% ± 0.9%). For the next 20 days, participants were provided a controlled diet containing either a standard [1.1 ± 0.0 g protein/kg per day ($n = 8$)] or higher amount of protein [2.1 ± 0.0 g protein/kg per day ($n = 9$)]. The standard protein level was chosen based on mean *ad libitum* protein intakes in active-duty military personnel (Margolis et al., 2012), and the higher protein level has been shown to attenuate fat-free mass loss during energy deficit at sea level (Pasiakos et al., 2013). Dietary protein had no effect on any outcome measure in this study (data not shown) and, therefore, marginal means for time are shown.

Blood collection and circulating biomarkers of iron homeostasis

Fasting blood samples were collected 30 minutes after ceasing supplemental oxygen (day 1) and after 20 days at

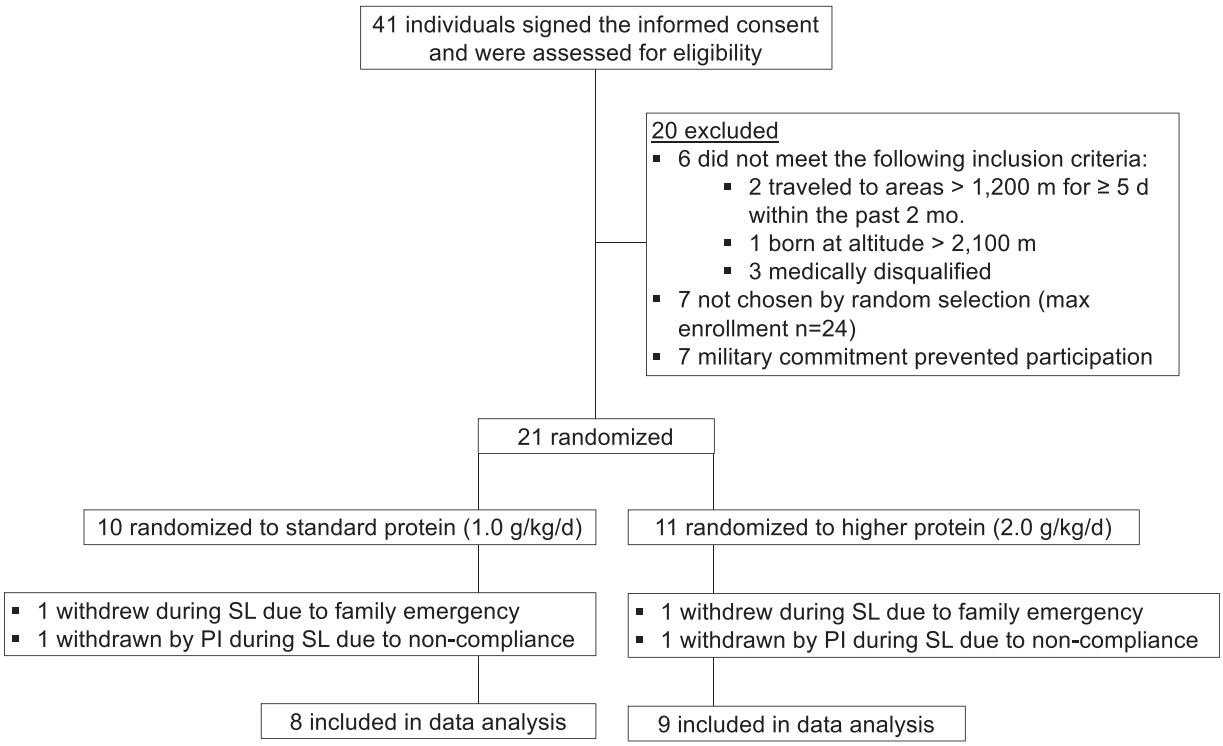

FIG. 1. Volunteer selection chart. PI, principal investigator; SL, sea level.

Table 1. Participant Characteristics at Sea Level and High Altitude

	Sea level	*HA*	p
Age, years	23.4 ± 5.6	—	
Weight, kg	82.9 ± 14.5	75.0 ± 13.2	<0.0001
BMI, kg/m^2	26.5 ± 3.7	24.0 ± 3.4	<0.0001
Body fat, %	23.0 ± 6.1	20.0 ± 8.3	0.0003
SpO_2, %	97.0 ± 0.9	87.0 ± 1.5	<0.0001
Dietary iron, mg/day	11.6 ± 4.1	12.0 ± 1.2	0.67
Energy, kcal/day	2394 ± 426	1916 ± 229	<0.0001

Values are means ± standard deviation. Average dietary iron and energy intakes for the 3-week period at sea level and HA are shown; all other values represent the final day at sea level or HA, respectively.

BMI, body mass index; HA, high altitude; SpO_2, peripheral capillary oxygen saturation.

HA (day 20). Twenty days was selected as the data point as hepcidin expression is lowest with chronic altitude exposure (i.e., >10 days) compared with acute altitude exposure (Piperno et al., 2011). Serum ferritin was measured using an automated immunoassay instrument (Siemens Medical Solutions USA, Inc.). Soluble transferrin receptor (sTfR) was determined using a Quantikine IVD ELISA Kit (R&D Systems Inc.). Serum iron and total iron-binding capacity (TIBC) were measured using the Beckman Coulter DxC 600 Pro System (Beckman Coulter), and transferrin saturation was calculated by dividing serum iron by TIBC. Hematocrit was determined in whole heparinized blood using a handheld iSTAT® point-of-care device and Chem8+ Cartridges (Abbott Laboratories). Serum hepcidin was assessed using enzyme-linked immunoassays (high sensitivity; DRG International).

Statistical analysis

Data are reported as means ± standard deviation. Comparisons between days 1 and 20 were conducted using paired Student's *t*-tests. Pearson correlations were conducted when testing for linear associations. Differences were considered significant if $p < 0.05$. Data were analyzed using SPSS version 21 (IBM Corp.) and graphed using GraphPad Prism 5.04 (GraphPad Software Inc.).

Results

Participants' demographics and dietary information after sea level and HA are given in Table 1. As reported previously, total energy deficit at HA was −1849 ± 511 kcal/day, inducing an overall weight loss of 7.9 ± 1.9 kg (Berryman et al., 2017). Dietary iron intake remained constant from sea level to HA. Twenty days at HA resulted in a 12% increase in hematocrit (Fig. 2A; $p < 0.01$) and a 37% decline in serum hepcidin concentrations (Fig. 2B; $p < 0.01$) compared to day 1. Serum iron (65.4 ± 16.5 and 104.7 ± 34.2 μg/dL; $p < 0.01$) and TIBC (317.2 ± 37.2 and 332.5 ± 30.0 μg/dL; $p < 0.01$) increased, resulting in a 55% increase in transferrin saturation (Fig. 2C; $p < 0.01$) after 20 days at HA compared to day 1. Ferritin concentrations declined by 18% (Fig. 2D; $p = 0.02$), sTfR increased by 83% (Fig. 2E; $p < 0.01$), and the sTfR to $\log_{10}$ ferritin ratio, an index of iron-dependent erythropoietic activity (Punnonen et al., 1997; Beguin, 2003), increased by 99% (Fig. 2F; $p < 0.0001$) on day 20 compared to day 1. Circulating concentrations of interleukin-6 were not different between days 1 and 20 at HA (11.0 ± 16.6 and 11.9 ± 16.7 pg/mL; $p = 0.31$).

Discussion

In this study, we report suppressed hepcidin concentrations and increased iron availability in lowlanders experiencing

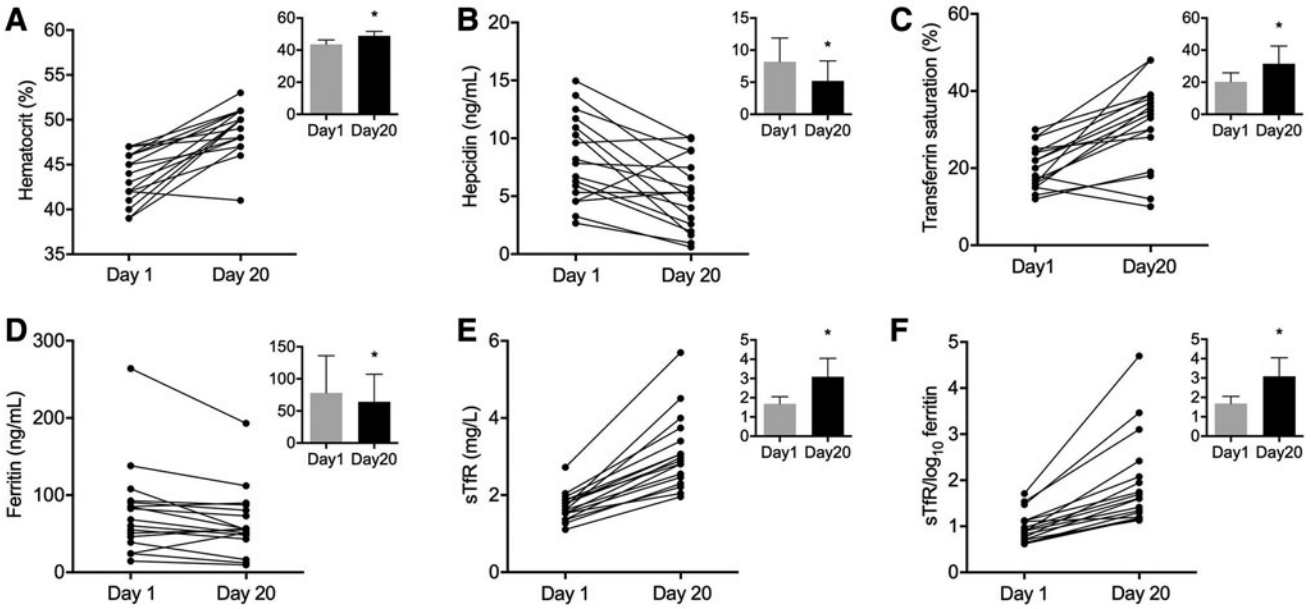

FIG. 2. Changes in hematocrit, hepcidin, and indicators of iron status during energy deficit at HA. Line graphs for each participant ($n = 17$) and means ± standard deviations are shown for **(A)** hematocrit and **(B)** hepcidin, **(C)** transferrin saturation, **(D)** ferritin, **(E)** sTfR, and **(F)** sTfR/$\log_{10}$ ferritin. An *asterisk* denotes a significant difference ($p < 0.05$). HA, high altitude; sTfR, soluble transferrin receptor.

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severe energy deficit during a 20-day sojourn to 4300 m above sea level. These data suggest that increased erythropoietic activity with HA acclimatization suppresses hepcidin expression to increase iron availability during severe energy deficit.

Dietary iron intake and body iron stores decline with exposure to hypoxia (Richalet et al., 1994; Robach et al., 2004). The former is due to a decrease in overall food intake, which may be explained by decreased appetite, nausea, and vomiting from acute mountain sickness, or a decreased availability of food (Butterfield, 1999). The latter may be explained by a reduction in dietary iron intake and/or iron mobilization to the erythropoietic compartment. The suppression in hepcidin signals for iron release from storage organs including macrophages and enhanced absorption of dietary iron in the intestine (Gassmann and Muckenthaler, 2015). In this study, the decline in ferritin and increase in sTfR suggest a reduction in body iron stores with hypoxia and energy deficit even when dietary iron intake was held constant. The increase in transferrin saturation was a result of increased serum iron and TIBC. In one study, after spending 3 days at 3400 m, participants trekked for a period of 5 days to 5400 m where they remained for 11 days (Piperno et al., 2011). Transferrin saturation decreased compared with that at sea level after 20 days at >3400 m, which was consistent with iron mobilization and transfer to the erythropoietic compartment. These seemingly contradictory findings may be due to the opposing effects of energy deficit and hypoxia on erythropoiesis and iron metabolism. Severe energy deprivation inhibits erythropoiesis (Fried et al., 1957; McCarthy et al., 1959; Donati et al., 1964; Fruhman, 1966), which is accompanied by decreases in erythroid progenitors in the bone marrow to preserve iron stores for vital functions. Regardless, these data indicate a strong overall iron mobilization and further support the concept of increased iron transfer to the erythropoietic compartment during energy deficit at HA.

Recent evidence in humans (Troutt et al., 2012) and rodent models (Mirciov et al., 2018) indicates that food deprivation increases hepcidin production. A time-dependent increase in serum hepcidin was observed in men and women during an 18-, 42-, and 66-hour fast compared with baseline (Troutt et al., 2012). Interestingly, hepcidin declined slightly, but remained elevated compared with baseline, when participants were refed with a small amount of food (500 kcals) in the 24 hours after fasting. Previous studies that have measured circulating concentrations of hepcidin at HA report a >80% reduction in hepcidin with exposure to HA (Piperno et al., 2011; Talbot et al., 2012; Goetze et al., 2013; Lundgrin et al., 2013). Although this study did not test the independent effects of energy deficit and hypoxia on hepcidin, the reduction in hepcidin with HA during energy deficit in this study was only 37% (i.e., >50% less than the other reports). Findings from this study suggest that the magnitude of the reduction in hepcidin at HA may be reduced with energy deficit, but that the increased erythropoietic activity with hypoxia may override the stimulatory effects of energy deficit on hepcidin expression.

The regulation of hepcidin in the context of hypoxia remains unclear. Piperno et al. (2011) observed a strong correlation between serum ferritin and hepcidin at HA and hypothesized that iron or the kinetics of iron use in response to hypoxia may signal for the downregulation in hepcidin (Piperno et al., 2011). Consistent with their findings, the percentage change in hepcidin during HA acclimation and energy deficit in this study was strongly correlated with the change in ferritin ($r = 0.894$, $p < 0.01$). However, Talbot et al. (2012) supplied volunteers with intravenous infusion of either 200 mg iron or saline placebo immediately before HA exposure and found that the suppression in hepcidin by hypoxia was too rapid to be due to a reduction in iron availability (Talbot et al., 2012), suggesting that a signal released during erythropoiesis and/or hypoxia may signal for the downregulation in hepcidin.

In this study, HA and energy deficit had no effect on circulating concentrations of interleukin-6, a known inflammatory regulator of hepcidin expression (Nemeth et al., 2004). Erythroferrone (ERFE) is a recently identified hormone encoded by the *FAM132B* gene that mediates hepcidin suppression during stress erythropoiesis (Kautz et al., 2014). Kautz et al. (2014) demonstrated that erythropoietin stimulates expression of ERFE in the bone marrow through Jak2-Stat5 signaling and that ERFE is secreted by (pro)erythroblasts to suppress hepcidin expression in mouse liver (Kautz et al., 2014). A validated commercially available kit to measure serum ERFE was not available at the time of this study; however, recently, Talbot et al. indicated no relationship between hepcidin and ERFE in HA natives undergoing venesection for chronic mountain sickness (Talbot et al., 2016). Rodents exposed to acute hypoxia (6–15 hours) showed a persistent downregulation in hepcidin and a transient increase in ERFE in the bone marrow (Ravasi et al., 2018), indicating that alternative mediators may be involved in maintaining the downregulation in hepcidin with hypoxia. Further studies are needed to clarify the role of ERFE in hypoxia-induced inhibition of hepcidin.

Collectively, these data indicate that hypoxia is a strong signal for iron mobilization to support iron transfer to the erythropoietic compartment. Importantly, dietary iron and iron recycling from macrophages cannot match the increased requirements for iron at HA. These findings are relevant to military personnel, who often endure sustained periods of energy restriction during training and combat operations in HA regions, for mountaineering expeditions and those trekking to HA, and may be clinically relevant to the hypoxemia and energy restriction observed in critically ill patients.

Authors' Contributions

S.R.H., C.E.B., A.J.Y., J.P.M., and S.M.P. conceived the study. S.R.H., C.E.B., B.J.A., A.J.Y., J.P.M., and S.M.P. conducted the study and A.M.K. and B.J.A. performed the experiments. S.R.H. analyzed the data and wrote the article with input from all authors. All authors have reviewed and approved the article.

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Author Disclosure Statement

The authors declare no competing financial interests.

Funding Information

This study was supported, in part, by the U.S. Army Medical Research and Material Command and appointment to the U.S. Army Research Institute of Environmental Medicine administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the U.S. Army Medical Research and Material Command.

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Received September 16, 2019;
accepted in final form March 11, 2020.

Testosterone Administration During Energy Deficit Suppresses Hepcidin Concentrations and Increases Iron Utilization in Healthy Males (OR15-08-19)

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Objectives: Military personnel are exposed to periods of energy deficit and stress, which is typically accompanied by reductions in testosterone concentrations and declines in iron status. Exogenous testosterone administration may increase erythropoiesis and iron availability.

The objective of this study was to determine the effects of exogenous testosterone on iron homeostasis in healthy young males during energy deficit.

Methods: Fifty males (24.8 ± 5.0 y, 78.6 ± 12.2 kg) completed this randomized, double-blind, placebo-controlled trial. Participants were provided a eucaloric diet during a 2-week free-living phase (day 1–14). Following the free-living phase, participants completed a 4-week live-in phase (day 15–42). During the live-in phase participants were randomized to 200 mg testosterone enanthate/week ($n = 24$) or 1 mL sesame oil/week (placebo, $n = 26$) and were in an energy- and exercise-induced energy deficit equal to 53 ± 4%. Blood was collected on days 14 and 42 to determine concentrations of testosterone, hemoglobin, hematocrit, ferritin, hepcidin, and erythroferrone. Data are reported as change scores (mean ± SD).

Results: Total testosterone concentrations increased with testosterone and declined with placebo (569 ± 271 nmol/L and −83 ± 142 nmol/L, $P < 0.0001$). Testosterone attenuated the decline in hemoglobin (−0.04 ± 0.7 g/L and −0.6 ± 0.8 g/dL, $P < 0.01$) and hematocrit (0.6 ± 2.2% and −1.3 ± 2.1%, $P < 0.01$) that occurred with placebo. Hepcidin (−3.7 ± 5.0 ng/mL and 2.1 ± 5.0 ng/mL, $P < 0.001$) and ferritin (−32.5 ± 38.6 ng/mL and 15.4 ± 41.2 ng/mL, $P < 0.0001$) were reduced with testosterone as compared to placebo. Erythroferrone (−0.5 ± 2.1 ng/mL and 0.5 ± 2.0 ng/mL, $P = 0.11$) did not differ when comparing testosterone to placebo.

Conclusions: Testosterone administration during energy deficit suppressed hepcidin concentrations and increased iron utilization in healthy young males. The reduction in hepcidin with testosterone administration appears to occur through an erythroferrone-independent mechanism.

Funding Sources: The views expressed are those of the authors and do not reflect the official policy of the Army, DoD, or the U.S. Government. Supported by the Collaborative Research to Optimize Warfighter Nutrition II and III projects and the Defense Health Program Joint Program Committee-5.

Military Dining Facility Staff Perspectives on the Implementation Barriers and Facilitators of Go for Green 2.0, a Department of Defense Nutrition Initiative

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Background: In an effort to optimize performance, readiness, and health of military service members, the Department of Defense (DoD) created a performance nutrition initiative, Go for Green (G4G). Challenges led to program revision and development of G4G 2.0, however successful implementation has yet to be accomplished.

Objective: The purpose of this study was to improve the G4G 2.0 program framework by informing and developing best practices through explication of dining facility (DFAC) staff perspectives.

Study Design, Settings, Participants: Focus groups were conducted in two phases (May 2017 to February 2018) with supervisors and staff of Wolf DFAC, at Fort Carson, Colorado prior to G4G 2.0 implementation. Participants were recruited by purposive sampling (n = 89). Focus groups were audio recorded to capture all verbal responses, transcribed verbatim, and lasted an average of 31 minutes (range 24 to 58 minutes).

Measurable Outcome/Analysis: Focus group transcripts of DFAC staff perspectives were reviewed via qualiP175 (continued) Continued on page S113 S112 Poster Abstracts Journal of Nutrition Education and Behavior Volume 51, Number 7S, 2019 tative content analysis using an exploratory, inductive approach to elucidate emergent themes from the data.

Results: DFAC staff perspectives of barriers to G4G implementation included lack of, or ineffective training, inconsistent preparation of food items, fidelity issues, staff perceptions of patron preferences, lack of patron familiarity to new G4G food items and lack of buy-in. Implementation facilitators included training, G4G team site visits, and patron exposure to G4G menu items. Staff suggestions for G4G implementation and program improvement included training on preparation methods, having a flexible implementation timeline, up front provision of all G4G materials, for the G4G team to conduct a needs assessment, and G4G and nutrition education and awareness outside the DFAC.

Conclusion: Results demonstrated that barriers to G4G 2.0 implementation outweighed facilitators, and supported the idea that there may be more viable, less time and work-intensive methods to promote healthier food selection by patrons. Incorporating methods to improve patron selection of healthful items in DFACs could play an instrumental role in improving Service Member health, thereby enhancing readiness to meet mission requirements.

Funding: United States Army Research Institute of Environmental Medicine.

Using the Healthy Eating Score-5 as a measure of diet quality in an Active Duty Military population

Rachel N. Dyal, Bethany A. Deschamps, Susan M. McGraw, Julianna M. Jayne, J. Philip Karl, Renee E. Cole

Abstract

Background: Having an accurate and time-efficient method of assessing diet quality is important to understand the nutritional health of a population, and to guide health promotion and dietary interventions. The five-question Healthy Eating Score-5 (HES-5) was developed to quickly assess diet quality in a military population in place of the 110-question Block food frequencies questionnaire (FFQ) and Healthy Eating Index (HEI) developed in 2005. Although the HEI was updated in 2015, the association with the HES-5 has not been re-assessed. This study aimed to determine the correlation between the HES-5 and the HEI-2015, and if that correlation could be strengthened by adding questions to the HES-5.

Methods: Soldiers (N = 446, 91% male, 89% enlisted, 23 ± 3 years old) completed the HES-5, five additional diet-related questions, and the validated Block FFQ. HEI-2015 scores were calculated from responses to the Block FFQ. The HES-5 asked respondents about their frequency of vegetable, fruit, whole grain, dairy, and fish consumption. Each category was scored on a 5-point scale with higher scores indicating more frequent consumption. The five additional questions assessed alcohol (ALC), energy drink (ED), sugar-sweetened beverage (SSB), and breakfast consumption (BC), and dining out (DO) frequency. ALC, ED, SSB, and DO were reverse scored. Various combinations of these questions were then added to the HES-

5. Normality was assessed, and Pearson's correlation was used to determine which combination of HES-5 and additional questions best correlated to the HEI-2015.

Results: Mean ± SD HEI-2015 scores were 59 ± 10 out of 100 points matching the average HEI-2015 score in the American population and reflecting poor diet quality. HES-5 scores averaged 11 ± 4 out of 25 points. The means of the highest and lowest quartile of HES-5 scores were 16 ± 2 and 6 ± 2 respectfully. Only 0.2% of the sample received a score of 25 which indicates meeting the dietary guidelines. There was as moderate, positive correlation between the original HES-5 and HEI-2015 ($r = 0.41$, N = 433, $p < 0.01$). Of the various HES question combinations assessed, this correlation increased the most with the addition of the SSB, DO and ED questions (HES-8; $r = 0.54$, N = 433, $p < 0.01$; mean ± SD 22 ± 5 out of 40 points).

Conclusion: Results indicate that, in our population, the correlation between the HES-5 and HEI-2015 was similar to previously reported correlations between the HES-5 and HEI-2015 ($r = 0.41$, N = 333, $p < 0.0001$). However, adding three questions related to SSB, ED, and DO strengthened the correlation between the HES-5 and the HEI-2015. The HES-8 therefore provides a better assessment of diet quality than the currently used HES-5. In future versions of the HES, rephrasing HES questions to target consumption amount instead of frequency may improve correlation with the HEI-2015 which is based on amount consumed. Research to assess the validity of the HES-8 using more precise dietary assessment methods is also needed.

Disclaimer: The views expressed in this abstract are those of the author(s) and do not reflect the official policy of the Department of the Army/Navy/Air Force, Department of Defense, or U.S. Government.

Funding Source: US Army Medical Research and Materiel Command

Emotional Eating Behavior Mediates the Association between Perceived Stress and Body Weight Status

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Abstract

Background: Overweight and obesity among Soldiers is a major concern for the US Army because it must maintain a healthy and fit fighting force. Research in civilian populations demonstrates overweight and obesity to be associated with eating in response to external cues, such as perceived stress, but this association has not been examined in Soldiers who face unique stressors and eating environments. This cross-sectional study aimed to examine the relationship between failing body composition (BC) standards, body mass index (BMI), perceived stress (PS), and emotional eating (EE) among Soldiers, and whether the effect of EE on study outcomes differed by sex. **Methods:** Soldiers (N=1,460, 83% male, 93% enlisted, 23.5 ± 5.2 years of age with 3.5 ± 4.0 years of service) from seven Army posts completed validated surveys that obtained information on eating behaviors or mediators of eating behaviors including, PS, EE, adherence with military BC standards, and sociodemographic information. Hierarchical logistic and linear multiple regression analysis was implemented to test EE as a mediator between PS and BMI, and between PS and BC failure. Sobel-Goodman test of mediation was used to determine the percentage of the relationship between study outcomes and PS explained by EE. **Results:** Mean ± SD PS score were 17.5 ± 4.7 and 17.7 ± 4.4 for men and women, respectively (p=0.46), indicating moderate PS. Sex differences in Soldiers reporting EE behaviors sometimes or often were significantly different at 29% and 44% of men and women, respectively (p<0.001). Higher PS was associated with more frequent self-reported EE behaviors (p<0.001), higher BMI (p<0.001), and higher odds of BC failure (95% CI: 1.02, 1.09). EE was a complete mediator in the relationship between BMI and PS and a partial mediator in the relationship between BC failure and PS. The effect of EE on BMI and BC failure was statistically significant with approximately 30% (p=0.01) and 35% (p<0.001) of the total effect being mediated, respectively.

Conclusions: EE behaviors fully explain the association between PS and BMI. PS was also associated with BC failure, with the odds of BC failure being higher among Soldiers who exhibited EE behaviors compared to those who did not. These findings suggest EE behaviors may be potential intervention targets within multidisciplinary behavioral health programs aiming to prevent and manage overweight and obesity among Soldiers.

The association between military weight status and body image in a sample of active duty Soldiers

Maria J. Stukenborg, Bethany A. Deschamps, Susan M. McGraw, Julianna M. Jayne, J. Philip Karl, Renee E. Cole

Background: Negative body image has been correlated with depression, unhealthy dieting behaviors, and elevated body mass index (BMI). Studies indicate that athletes in sports with body composition requirements often report increased body dissatisfaction. Soldiers are likewise required to meet body composition and physical fitness standards during their time in service. However, the impact of military weight status on body image among Soldiers has not been thoroughly studied. This study aimed to identify the association between military weight status, as measured by compliance with body composition standards, and time in service with body image among Soldiers.

Methods: Surveys were administered to active-duty Soldiers (N=969, 86% male, 91% enlisted, 23.5 ± 5.2 years of age, 3.5 ± 4.2 years of service) to collect data on military weight status, time in service, body image, and demographics. Twelve body image questions were rated on a 5-point Likert-type scale wherein higher scores represented a more unfavorable body image. In statistical models, body image questions were grouped together (12 items; α=0.88) and Soldiers were dichotomized into 2 groups based on the mean of the scores: less-favorable (≥ 35.5 points) and favorable (< 35.5 points body) image groups. Military weight status was determined by whether a Soldier had ever failed a body fat tape test during their military career. Multivariate logistic regression models were used to examine the relationship between military weight status, time in service, and body image with the covariates of sex, education, marital status, current duty station, race/ethnicity, and rank.

Results: The mean ± SD total body image score was 35.5 ± 8.2 and was normally distributed. Soldiers who reported having failed the body fat tape test (N=148, 25.5 ± 5.6 years of age, BMI of 29.5 ± 3.1) were 5.65 times ($p < 0.001$; 95% CI: 3.30, 9.70) more likely to have a less-favorable body image than those who had never undergone the body fat tape test (N = 138, 23.5 ± 5.3 years of age, BMI of 23.5 ± 2.4). Soldiers who had been taped but never failed the test (N = 659, 23.8 ± 5.5 years of age, BMI of 25.7 ± 3.3) were 1.51 times ($p = 0.034$; 95% CI: 1.03, 2.22) more likely to have a less-favorable body image than those who had never undergone the body fat tape test. There was not a significant relationship between time in service and body image.

Conclusion: Findings support an association between undergoing the body fat tape test and a less-favorable body image among Soldiers. Since negative body image in civilian populations has been linked to increased depression, undesirable eating behaviors, and a higher BMI, further studies examining the effects of body composition standards on Soldiers' body image are warranted.

Current Word count: 448

Disclaimer: The views expressed in this abstract are those of the author(s) and do not reflect the official policy of the Department of the Army/Navy/Air Force, Department of Defense, or U.S. Government.

Funding Source: US Army Medical Research and Materiel Command

NOTE: MHSRS Requirements:
Title restricted to 255 characters
Abstract restricted to 500 words

Does Go For Green 2.0 Joint Service Performance-Nutrition Initiative Improve Dietary Intake of Soldiers?

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Background: Poor diet quality may contribute to increased military overweight rates and associated comorbidities. Go For Green® version 2.0 (G4G 2.0) is a project to improve soldiers' food environment to optimize performance, readiness, and health. Objective - to determine if soldiers' diet quality at baseline improved after implementation of G4G 2.0 at a Colorado military dining facility (DFAC).
Methods: Soldiers recruited at baseline (n=89) and post G4G 2.0 implementation (n=45) were asked to consume three meals in the DFAC over a 2-day period per iteration. Soldiers consuming only one meal were excluded. Soldiers' before/after dining trays were captured using digital photography. Foods consumed, estimated by two trained dietitians, were analyzed for nutrient content (FNDDS 2015-2016). Healthy Eating Index-2015 (HEI-2015; 100 points maximum score), diet quality measure reflecting the 2015-2020 Dietary Guidelines for Americans, was calculated for soldiers consuming at least 2 different meals. Data analysis used SAS® version 9.4 software.
Results: At baseline, HEI-2015 total score was 62.9 ± 9.0 pts (n=75). Lowest component score was whole grain (0.45 ± 1.5 pts); total protein was 94.0% of maximum score, and total fruits, vegetables and dairy were 69.6%, 77.7%, and 63.3% of target, respectively. After G4G 2.0 implementation, mean HEI-2015 total score was 71.2 ± 10.9 pts (n=40, $p < 0.001$). Whole grain consumption increased (3.9 ± 2.9, $p<0.001$), protein was 97.5% of maximum score, and total fruits, vegetables and dairy were 68.1%, 79.1%, and 72.0% of target, respectively.
Conclusions: Soldiers tasked with defending our nation should be in optimal nutritional status. Low baseline diet quality scores supported need for a DFAC nutrition intervention aimed to improve food offerings and soldier nutrient intake. These results suggest that G4G intervention can help to increase diet quality in military dining facilities.

Disclosure: Views expressed do not reflect Dept. of Army/Defense or U.S. Government official policy.

126 May 27 10:15 AM - 10:30 AM

Testosterone Supplementation Upregulates Myogenesis And Attenuates Proteolytic Gene Expression During Severe Energy Deficit

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(No relationships reported)

BACKGROUND: Testosterone supplementation promotes whole-body lean mass accretion during severe energy deficit in non-obese, young men. The intramuscular mechanisms mediating this effect remain undefined.
PURPOSE: Determine the effect of supplemental testosterone on androgen receptor (AR), anabolic, proteolytic, inflammatory, and myogenic pathways during energy deficit.
METHODS: Fifty men (mean ± SD; 25 ± 5 y, 25 ± 3 kg/m^2) completed a 14-d weight maintenance (WM) phase, followed by a 28-d, 55% diet and exercise-induced energy deficit (ED) with 200 mg testosterone enanthate/week (TEST, n = 24) or placebo (PLA, n = 26). Muscle biopsies (vastus lateralis) from a subset (n = 10 per group) of participants exhibiting the greatest increase or decrease in leg lean mass (DEXA) and total testosterone were assayed to assess phosphorylation status, total protein and gene expression using Western blotting and RT-qPCR. Biopsies were collected at the end of WM and ED at baseline, and at 1 and 6 h after exercise (1 h cycle ergometry matched between phases for power and total work), with a mixed-meal (40 g protein) consumed following the first post-exercise biopsy.
RESULTS: Change (ED – WM) in leg lean mass (1.2 ± 0.7 vs. -0.7 ± 0.4 kg) and total testosterone concentrations (712 ± 159 vs. -193 ± 78 ng/dL) differed between TEST and PLA (P < 0.05). Relative to WM, baseline AR total protein was 118 % higher for TEST than PLA (P < 0.05) and was associated with changes in leg lean mass and fibroblast growth factor-inducible 14 (Fn14) gene expression (r = .540 and -.563, P < 0.05). Baseline Fn14 gene expression relative to WM was 52 % lower for TEST than PLA (P < 0.05) and tended to associate with changes in leg lean mass (r = -.455, P = 0.058). After exercise (6 h), muscle atrophy F-box and muscle ring finger-1 gene expression was lower (61 and 44 %) and MyoD expression was 122 % higher for TEST than PLA (P < 0.05), relative to WM. Mechanistic target of rapamycin-mediated anabolic signaling was not different between groups.
CONCLUSIONS: The hypertrophic effect of testosterone on lean mass during severe energy deficit may be due, in part, to reduced ubiquitin-mediated proteolysis and increased myogenesis after exercise and feeding, subsequent to modulation of upstream AR and Fn14 signaling. Supported by USAMRDC; authors' views not official U.S. Army or DoD policy.

(P10-042-20) Effects of Testosterone on Mixed Muscle Protein Synthesis and Proteome Dynamics During Energy Deficit

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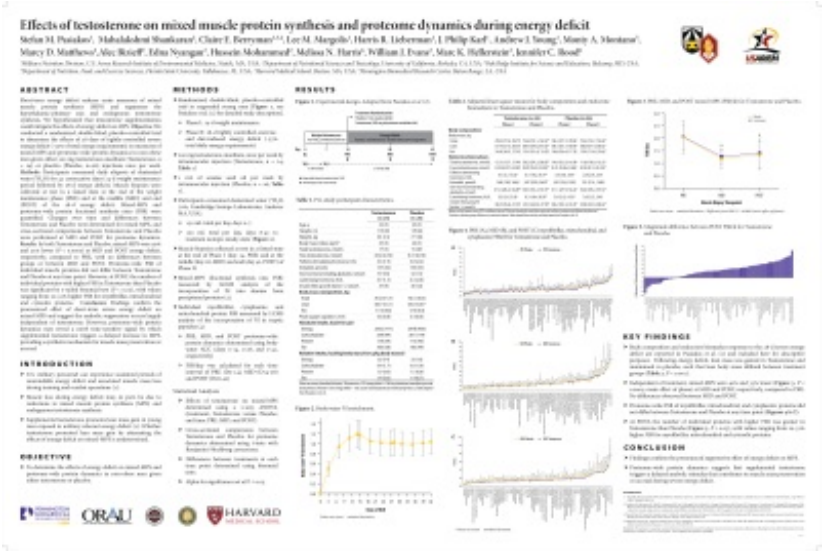
Topical Area: Energy and Macronutrient Metabolism



Objectives: Short-term energy deficit reduces acute measures of mixed muscle protein synthesis (MPS) and suppresses the hypothalamic-pituitary axis and endogenous testosterone synthesis. We hypothesized that testosterone supplementation could mitigate the effects of energy deficit on MPS. We conducted a randomized, double-blind, placebo-controlled trial to determine the effects of 28 days of tightly-controlled severe energy deficit (deficit 55% of total energy requirements) on measures of mixed-MPS and proteome-wide protein dynamics in non-obese men either given 200 mg testosterone enanthate (Testosterone, n = 24) or placebo (Placebo, n=26) injections per week.

Methods: Participants received daily aliquots of deuterated water (^2H_2O) for 42 consecutive days (14-d weight maintenance period followed by 28-d energy deficit). Muscle biopsies were collected at rest in a fasted state at the end of the weight maintenance phase (PRE) and at the middle (MID) and end (POST) of the 28-d energy deficit. Mixed-MPS and proteome-wide protein fractional synthesis rates (FSR) were quantified. Changes over time and differences between Testosterone and Placebo were determined for mixed-MPS, and cross-sectional comparisons between Testosterone and Placebo were performed at MID and POST for proteome dynamics.

Results: In both Testosterone and Placebo, mixed-MPS were 40% and 33% lower (P < 0.0005) at MID and POST energy deficit, respectively, compared to PRE, with no differences between groups or between MID and POST. Proteome-wide FSR of individual muscle proteins did not differ between Testosterone and Placebo at any time point.

However, at POST, the number of individual proteins with higher FSR in Testosterone than Placebo was significant by 2-tailed binomial test ($P < 0.05$), with values ranging from 20-32% higher FSR for myofibrillar, mitochondrial and cytosolic proteins.

Conclusions: Findings confirm the pronounced effect of short-term severe energy deficit on mixed-MPS and suggest the anabolic suppression occurs largely independent of testosterone. However, proteome-wide protein dynamics may reveal a novel time sensitive signal by which supplemental testosterone triggers a delayed increase in MPS, providing a synthetic mechanism for muscle mass preservation or accrual.

Funding Sources: Supported by DHP JPC-5/MOMRP; authors' views not official U.S. Army or DoD policy

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(P10-007-20) Longitudinal Comparison of Lean Body Mass by Dual Energy X-Ray Absorptiometry and Muscle Mass by Creatine (methyl-d3) Dilution in Response to a 28-d Severe Energy Deficit

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Topical Area: Energy and Macronutrient Metabolism

Objectives: We reported that healthy males supplemented with testosterone gained lean body mass (LBM) during 28-d of energy deficit and 14 d of ad libitium feeding when measured by dual energy x-ray absorptiometry (DXA), but with no increase in muscle strength. We were unable to determine whether LBM gains were due to muscle mass accrual since DXA does not deliniate muscle from visceral organs and body water. **Objectives:** To assess the effects of testosterone supplementation on muscle mass as measured by creatine (*methyl*-d_3) dilution, and determine the relationship between muscle mass and DXA-measured LBM in response to short-term energy deficit.

Methods: Secondary analysis of a 3-phase, randomised, double-blind, placebo controlled trial in healthy males: 14-d free-living, eucaloric phase (P1); 28-d live-in, 55% energy deficit phase with (200 mg testosterone enanthate/wk, TEST, n = 24) or without (PLA, n = 26) testosterone (P2); and 14-d free-living, ad libitum feeding phase (P3). Muscle mass was measured by creatine dilution and LBM by DXA at the end of each phase.

Results: We previously reported increased LBM in TEST (mean change from P1 ± SEM; 2.5 ± 0.4, $P < 0.01$), but not PLA (-0.3 ± 0.3 kg, $P > 0.05$), following P2. Both TEST (5.2 ± 0.4 kg) and PLA (2.2 ± 0.4 kg) gained LBM in P3 ($P < 0.01$). There was a treatment-by-phase trend for change in muscle mass (P-interaction = 0.054), but muscle mass data were highly variable and no post-hoc comparisons met statistical significance (PLA, P2: -0.3 ± 1.6; TEST, P2: -0.7 ± 1.7; PLA, P3: -0.3 ± 1.6; TEST, P3: 3.8 ± 1.7 kg; $P > 0.05$). Cross-sectional measures of muscle mass were correlated ($P < 0.001$) with total and appendicular LBM at P1 (r = 0.63 and 0.67), P2 (r = 0.71 and 0.72), and P3 (r = 0.55 and 0.48), respectively. However, changes in muscle mass were not associated with changes in total or appendicular LBM at P2 or P3 ($P > 0.05$).

Conclusions: Testosterone supplementation increased LBM in response to short-term energy deficit and recovery feeding, but had no significant effect on muscle mass or muscle function. The discordance between changes in LBM and muscle mass underscore the inherent limitations of LBM as a surrogate measure for skeletal muscle mass, particularly in response to short-term intervention studies.

Funding Sources: DHP JPC-5/MOMRP; authors' views not official U.S. Army or DoD policy.

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