Protocol Title: Physiological and psychological effects of testosterone during severe energy deficit and recovery: a randomized, placebo-controlled trial

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IRB Review History

Executive Summary

Energy deficit during military operations is often ~50% of total daily energy requirements and can reach near complete starvation.[1,2] The physiological consequences of severe energy deficit include physical performance decrements, particularly energy deficit-induced hypogonadism and the loss of lean body mass, which cannot be overcome with macronutrient modification alone. [3-7] Prolonged energy deficit also impacts mood, attentiveness, and decision-making capabilities. The extent to which energy deficit-induced hypogonadism contributes to physiological and psychological declines during sustained energy deficit remains poorly understood. Therefore, the objective of this study is to determine whether maintaining a eugonadal state during severe, sustained energy deficit attenuates physiological decrements, particularly the loss of lean body mass, and maintains mental performance. To address these objectives and more (e.g., gut health, appetite regulation, physiological and psychological recovery), we will enroll up to 60 physically active men in a 3-phase, randomized, placebo-controlled study. After completing a 14-day (**free-living, phase 1**), energy-adequate, diet acclimation phase (protein, 1.6 $g \cdot kg^{-1} \cdot d^{-1}$; fat, 30% total energy intake; with remaining energy derived from carbohydrate), participants will be randomized to one of two experimental groups and undergo a 28-day (**live-in, phase 2**), 55% energy deficit phase: energy deficit alone (DEF) or energy deficit + exogenous testosterone (DEF+TEST). Recovery (**free-living, phase 3**) will be assessed after completing phase 2 to determine when body mass has been recovered within ± 2.5% of initial body mass (duration will vary, 42-day maximum for phase 3). Body composition, state-of-the-art stable isotope methodologies, proteomics, metabolomics, muscle biopsies, whole-room calorimetry, molecular biology, activity/sleep monitoring, personality and cognitive function

assessments, functional MRI (fMRI), biochemistries, and rigorously controlled diet and physical activity will be used to assess physiological and psychological responses to energy restriction and recovery feeding while in a hypogonadal versus eugonadal state. Results of the proposed experiments will provide a basis for future studies aimed at improving Warfighter resiliency to and recovery from negative energy balance.

Statement of Need
There is a critical need for effective and feasible interventions that sustain and optimize Warfighter health and performance during real-world training and combat operations. This study will delineate the contribution of testosterone declines from the physical and mental demands encountered by Warfighters during military training and combat operations on complex markers of physiological and psychological status, addressing a direct, consistently observed gap in knowledge.

Objective I: Effects of exogenous testosterone during energy deficit and recovery.

A. Determine the extent to which maintenance of a eugonadal state by exogenous testosterone administration attenuates the effects of severe, sustained energy deficit on body composition (body mass, lean body mass and fat mass), skeletal muscle (mass, strength/power/endurance, proteomics, intramuscular regulators of metabolism, protein synthesis and proteolysis), metabolism (energy expenditure, substrate oxidation and nitrogen balance) and physiological status (androgens, stress and metabolic hormones, inflammation, hepcidin, iron status, circulating and intramuscular substrates and blood lipids).
B. Determine the effects of exogenous testosterone administration during severe, sustained energy deficit on subsequent recovery of body composition, skeletal muscle, and metabolic and physiological status.
C. Identify metabolomic and genetic biomarkers associated with habitual dietary intake and severe, sustained energy deficit and their predictive association with body composition, skeletal muscle, metabolism, and physiological status.
D. Determine the extent to which exogenous testosterone administration modulates both metabolomic biomarkers and associations between the metabolome and diet, body composition, skeletal muscle, metabolism, and physiological status during severe, sustained energy deficit.

Hypotheses for Objective I:

A. Severe energy deficit will elicit a hypogonadal state and loss of total body mass, lean body mass, and fat mass; exogenous testosterone administration will attenuate the loss of lean body mass and skeletal muscle mass and enhance the loss of body fat. In addition, during severe energy deficit, protein (e.g., glycolytic, mitochondrion, muscle development, actin cytoskeleton, etc.) synthetic rates will be downregulated, whereas intramuscular proteolysis will be upregulated, resulting in a loss of skeletal muscle mass and diminished muscular strength, power, and endurance; exogenous testosterone administration will maintain or upregulate synthesis rates relative to pre-deficit levels, particularly protein synthetic rates of contractile proteins. Exogenous testosterone administration will preserve indices of muscular strength, power, and endurance during severe energy deficit. Energy expenditure will be downregulated by energy restriction, with an increased reliance on endogenous protein for energy metabolism;

exogenous testosterone administration will maintain measures of physiological status and energy expenditure and shift substrate oxidation to promote a greater reliance on body fat.

B. Severe, sustained energy deficit and associated hypogonadism will impact recovery, as hypogonadal individuals will gain a disproportionate amount of body fat relative to lean body mass upon refeeding, resulting in an incomplete recovery of skeletal muscle strength, power, and endurance. Exogenous testosterone administration during severe, sustained energy deficit will limit fat accretion and promote gains in lean body and skeletal muscle mass while eating ad libitum in recovery.
C. Genetic polymorphisms and specific circulating metabolites will be associated with dietary intake, body composition, skeletal muscle, metabolic, and physiological status responses to severe, sustained energy deficit and recovery.
D. Exogenous testosterone administration will elicit a metabolomic signature indicative of sustained muscle anabolism that is associated with a preservation of muscle mass, function, and physiological status.

Objective II: Effects of severe energy deficit and exogenous testosterone on personality, mood, and cognition, and their association with genetic markers of brain function, stress, and endocrine function.

A. Determine the effects of severe, sustained energy deficit and associated hypogonadism on mental fatigue and other aspects of mood, cognitive performance, brain function and sleep.
B. Identify metabolic biomarkers of fatigue and their association with cognitive performance and sleep during metabolic stress using global metabolomics.
C. Determine the extent to which the detrimental effects of sustained energy deficit on mood, cognitive performance, and sleep are attenuated by pharmacological testosterone treatment.
D. Determine the extent to which exogenous testosterone administration modulates associations between the metabolome, fatigue, and cognitive performance during sustained energy deficit.
E. Identify genetic markers of mood, personality, cognitive performance, and sleep that predict, in part, responses to energy deprivation and testosterone treatment.

Hypotheses:

A. Stress-induced hypogonadism (i.e., energy deficit induced by sustained/ increased physical activity and inadequate dietary intake) will increase fatigue and diminish mental performance.
B. Metabolic profiles and specific circulating metabolites will be associated with hypogonadism, fatigue, and measures of mental performance during sustained energy deficit.
C. Exogenous testosterone administration during sustained energy restriction will attenuate fatigue, prevent temporary alterations in personality characteristics, and protect against decrements in mental performance.
D. Exogenous testosterone administration during sustained, severe energy restriction will elicit a metabolomics signature indicative of enhanced central nervous system (CNS) function that is associated with lower fatigue and improved mental performance.

E. Polymorphisms in CNS, stress, and endocrine genes will be associated with energy deprivation and exogenous testosterone administration.

Objective III-A: Effects of energy deficit and exogenous testosterone on appetite regulation.

A. Determine the effect of testosterone maintenance on appetite and adaptive responses of appetite-mediating hormones during energy deficit and body mass recovery in non-obese adults.

Hypotheses:

A. During energy deficit, leptin concentrations will decrease while appetite and acyl ghrelin concentrations will increase. Following weight regain, hormone concentrations will not differ from pre-energy deficit concentrations.
B. Testosterone maintenance will augment the energy deficit-mediated decrease in leptin and increase in ghrelin concentrations but will not affect appetite.

Objective III-B: Effects of energy deficit and exogenous testosterone on the gut microbiome and intestinal permeability.

A. Determine the effects of energy deficit with and without testosterone treatment on gut microbiota composition, function, and activity.
B. Identify associations between gut microbiota composition and function, host energy/substrate metabolism, body mass change, and the composition of body mass loss and regain.

Hypotheses:

A. Energy deficit will have a detrimental impact on the gut as evidenced by reduced fecal short chain fatty acid (SCFA) concentrations, decreased gut bacteria diversity, increased relative abundance of pro-inflammatory and mucolytic bacteria, and increased intestinal permeability. Body mass regain will reverse these effects.
B. Testosterone treatment will mitigate the unfavorable effect of energy deficit on the gut microbiome and intestinal permeability.

Background

Objective I: Effects of exogenous testosterone during energy deficit and recovery.

Strenuous work and inadequate energy intake during military operations produce severe energy deficits, depleted body energy stores, muscle mass loss (primarily lean body mass), degraded performance, and increased injury risk.[8-12] The physiological consequences of military operations occur to varying degrees.[1] During sustained military training operations lasting 3.5-64 days, Warfighters have experienced body mass losses ranging from 3-16% of initial body mass, with lean body mass accounting for, on average, more than 50% of the total body mass lost. [3,13-21] Decrements in total body and lean body mass generally occur with concomitant reductions in circulating levels of anabolic hormones (e.g., testosterone).[5]

Recent attempts to attenuate lean body mass loss induced by military operations have largely focused on dietary protein manipulations given that higher-protein diets have consistently been shown to spare lean body mass and maintain muscle anabolic sensitivity during moderate energy deficit.[22-24] For example, physically active adults consuming 1.6 and 2.4 $g \cdot kg^{-1} \cdot d^{-1}$during a 21-day, 40% energy deficit lost more body fat, spared more muscle mass, and maintained muscle protein synthetic responses to feeding to a greater extent than those who consumed 0.8 $g \cdot kg^{-1} \cdot d^{-1}$.[24] There were also no differences between those consuming 2.4 $g \cdot kg^{-1} \cdot d^{-1}$and 1.6 $g \cdot kg^{-1} \cdot d^{-1}$, suggesting that during moderate, sustained energy deficit, there are no advantages of consuming protein beyond twice the RDA.[24,25] These findings, which were derived from a well-controlled clinical trial, were used to develop operationally relevant dietary protein recommendations.[26] However, consuming approximately 1.6 $g \cdot kg^{-1} \cdot d^{-1}$ has generally been overwhelmed by the effects of severe energy deficit on protein retention during real-world military operations.[27]

We suspect that the dramatic reductions in testosterone that occur during severe energy deficit could diminish the efficacy of manipulating protein intake for sparing lean body mass during military operations. In healthy young males, the suppression of endogenous testosterone production has myriad adverse physiological consequences, including reduced lean body mass, increased adiposity, and decreased muscle strength.[28-31] Finkelstein et al. [29] recently demonstrated that decreased testosterone levels (from 530 to 350 $ng \cdot dL^{-1}$), achieved by goserelin acetate administration to reduce endogenous testosterone and estradiol production, result in increased adiposity, and further reductions to ≤ 200 $ng \cdot dL^{-1}$ are accompanied by skeletal muscle atrophy and decreased muscle strength. Importantly, testosterone decreases of this magnitude occur during military training and sustained operations, and are associated with concomitant decreases in lean body mass.[3-7] ***Although dietary macronutrient manipulations, to date, have proven unsuccessful at mitigating the endocrine response to severe negative energy balance,[32] pharmacologic interventions that restore anabolic hormone concentrations have been shown to promote nitrogen retention despite energy deficit.***[33-35] Whether preventing the decline in testosterone during conditions simulating severe, sustained operational stress enhances the protein-sparing effects of consuming a higher-protein diet has not been studied.

Although we suspect that maintaining testosterone production during severe, sustained energy deficit would attenuate the loss of lean body mass, the potential influence of testosterone maintenance on physiological and body composition in recovery from operational stress remains unclear. In general, refeeding following energy deficit is marked by the preferential accumulation of adipose tissue and not lean body mass.[36] This phenomenon of "rebound fatness" has been documented in Soldiers recovering from the sustained energy deficit experienced during US Army Ranger Training.[6] It is possible that the loss of body fat during energy deficit elicits a persistent suppression of metabolic rate during recovery, whereas the reductions in lean body mass promote hyperphagia in recovery from energy deficit.[37] Thus, we suspect that if testosterone levels are maintained during severe energy deficit, lean body mass will be spared, reducing subsequent hyperphagia and relative fat mass gain during refeeding and thereby setting the conditions for a more favorable recovery.

Results from the Functional Single Nucleotide Polymorphisms Associated with Human Muscle Size and Strength (FAMuSS) study suggest that genetic variants dictate individual skeletal muscle phenotypic and adaptive responses to stress (e.g., exercise training).[38] These findings suggest certain polymorphisms may predict skeletal muscle responses to underfeeding and testosterone treatment. More specifically, multiple genes involved in the regulation of muscle structure, growth, and inflammation were associated with sex-specific genetic skeletal muscle predisposition. Additional genes associated with body composition and skeletal muscle phenotype were highly predictive of whether an individual favorably responded to an exercise training stimulus. This study will therefore examine associations between genetic polymorphisms and body composition outcomes.

Objective II: Effects of severe energy deficit and exogenous testosterone on personality, mood, and cognition, and their association with genetic markers of brain function, stress, and endocrine function.

Testosterone appears to have a variety of effects on cognition including certain aspects of performance, mood, and sleep. Studies have reported linear[39-41] or nonlinear[42,43] relationships between serum testosterone levels and cognitive ability that suggest a U-shaped relationship between testosterone and performance.[44] Spatial abilities, verbal learning and memory, and nonverbal learning and memory all appear to be affected.[39-43] The literature also suggests a relationship between testosterone and socioemotional and economic behavior. High levels of endogenous testosterone have been associated with a greater tendency to pursue reward while ignoring potential threat,[45,46] and it has been shown that competitive success causes an increase in testosterone that encourages subsequent risk-taking behavior.[47] In men with low testosterone, supplementation exerts positive effects on mood and sexual behavior, reducing fatigue and depression and increasing self-esteem.[48-52] The literature also suggests that testosterone levels may impact behavioral characteristics such as the degree of masculine identification, social propensity, trust in others, and willingness to cooperate. However, relatively little work has been conducted to examine the impact of testosterone on brain function, cognition, and mood in healthy young men.

Beyond the apparent positive impact of testosterone on psychological status, testosterone administration may alter certain aspects of collaborative behavior by inducing individuals to increase the weight they assign to their own personal judgments relative to opinions of others.[53] Although testosterone does not appear to interfere with personal decision-making, its tendency to increase dominance and status-seeking behavior evidently promotes a degree of assertiveness. Zak et al.[54] found that subjects who had high levels of testosterone were less generous overall and more likely to punish ungenerous participants. Conversely, men with the lowest levels of testosterone were nearly 6 times more generous than their high-testosterone counterparts. These results are consistent with those from retrospective studies that have indicated high testosterone levels are associated with increased selfishness and a greater propensity to punish people who violate social norms.[55] They also are generally consistent with the idea that high testosterone is associated with dominance and aggression, as well as with a lack of trust and an increased responsiveness to perceived anger in others.[56,57]

Overall, published studies suggest testosterone has the potential to augment cognitive performance and mood and enhance assertiveness and risk-taking; however, much of the existing information is not definitive since it is based on correlational data, on subjects who may be anabolic steroid abusers, or on older individuals who are suffering from age-related hormonal changes. Testosterone treatment during a period of sustained energy deficit may reduce the adverse effects on mood associated with low testosterone levels. With regard to the sleep-disrupting (or possibly even sleep-improving) effects of either condition, additional research is essential since the available data also are confounded by the factors mentioned above.

The effect of testosterone supplementation on sleep quantity and quality has not been examined, but there may be a relationship between endogenous testosterone levels and sleep duration, as well as between endogenous testosterone and the length of wakefulness. An age-related decline in testosterone secretion has been observed, and it is thought this may be associated with age-related reductions in sleep quantity and quality.[58,59] With regard to testosterone supplementation, testosterone and anabolic steroids have been associated with reductions in sleep duration, increased insomnia, and sleep fragmentation, and several investigators have examined the relationship between testosterone and obstructive sleep apnea (OSA). While it has been concluded that OSA patients with low testosterone levels may experience sleep-related benefits from testosterone supplementation, little is known regarding the effects of testosterone on the sleep of normal young adults.[60]

Behaviors known to be associated with testosterone that will be assessed in this study, such as interpersonal trust, competition, and aggression, are known to be heritable and associated with polymorphisms in particular genes such as AVPR1a, OXTR, and HRT2A (genes coding respectively for arginine vasopressin, oxytocin, and the serotonin 2A receptor). At least 40% of the variability in testosterone-related behavioral functions may be explained by genetic factors.[61] In addition, it is well-established that a substantial proportion of individual variation in cognitive, emotional, and physical performance in response to stress is attributable to genetic differences. For example, 60% of the variance in the glucocorticoid response to stress may be due to genetic factors.[62] A variety of mechanisms may be responsible for differences in stress sensitivity, including the modulation of HPA axis sensitivity and variation in stress-induced neurotransmitter levels and receptor sensitivity.[63] For example, a single nucleotide polymorphism (SNP) within the GABRA6 gene (T1521C), a receptor for the inhibitory neurotransmitter GABA, is associated with blunted ACTH, cortisol, diastolic blood pressure, and mean blood pressure response to the Trier psychological stress test.[64] Similarly, repeat nucleotide polymorphisms in the gene encoding for tyrosine-hydroxylase (TCAT) are associated with differences in catecholamine secretion and cardiovascular reactivity to stress (Zhang et al., 2004).[65] Therefore, we will investigate polymorphisms associated with testosterone responsiveness, stress, and endocrine and brain function.

Objective III-A. Effects of energy deficit and exogenous testosterone on appetite regulation.

The appetite-mediating hormones, including anorexigenic hormones peptide-tyrosine tyrosine (PYY) and glucagon-like peptide-1 (GLP-1) and the orexigenic hormone ghrelin, are thought to contribute to the drive to eat and hunger after weight loss and may underlie the common tendency for weight regain.[66] However, supporting evidence is predominantly derived from studies conducted in obese populations. Given the high prevalence of dieting for weight loss and body weight cycling in non-obese populations, including Warfighters, and the increased risk of obesity that accompanies these behaviors,[67] improving current understanding of adaptive responses of appetite-mediating hormones to weight loss and weight regain in non-obese populations is imperative.

Of particular interest is the hormone ghrelin. Ghrelin is the only known orexigenic hormone to be secreted from the gastrointestinal tract. Current evidence indicates that ghrelin concentrations increase during energy deficit, but the evidence is limited to mainly studies that have measured only total (inactive + active) ghrelin concentrations. However, ghrelin circulates in both an inactive form (des-acyl ghrelin) and an active, appetite-stimulating form (acyl ghrelin).[68] Evidence from our group (O'Connor et al., unpublished data) and others[69] suggests that in non-obese adults, des-acyl and acyl ghrelin concentrations may respond differently from one another to acute energy deficit. These studies have employed near total energy restriction, which may prevent the conversion of des-acyl ghrelin to acyl ghrelin. The extent to which differences in des-acyl and acyl ghrelin responses are influenced by the magnitude of energy deficit and whether differential responses are extant over prolonged energy deficit have not been characterized in non-obese adults. This study will characterize adaptive responses of appetite mediating hormones in non-obese adults during energy deficit and determine whether these responses persist after body weight is restored to pre-intervention levels. Both des-acyl and acyl ghrelin will be measured to provide novel insight into the regulation of ghrelin activity in response to weight loss and regain in non-obese adults.

Hypothalamic-pituitary-gonadal axis function is one potential, but understudied, factor in adaptive responses of appetite-mediating hormones to energy deficit. Estrogens have been shown to modulate appetite-mediating hormone secretion and CNS sensitivity to gastroenteropancreatic hormones in both murine models and human studies.[70] Comparatively little is known about the effects of androgens on appetite-mediating hormones and eating behavior. Testosterone and ghrelin concentrations are positively associated in healthy men,[71] and testosterone therapy for hypogonadism has been accompanied by increases in ghrelin[72] and decreases in leptin concentrations.[73] This study will expand the current evidence base by determining to what extent testosterone maintenance alters appetite-mediating hormone concentrations and appetite during energy restriction and refeeding. Moreover, it will provide novel information regarding the role of testosterone on acyl ghrelin responses to energy availability and subsequent effects on appetite.

Objective III-B. Effects of energy deficit and exogenous testosterone on the gut microbiome and intestinal permeability.

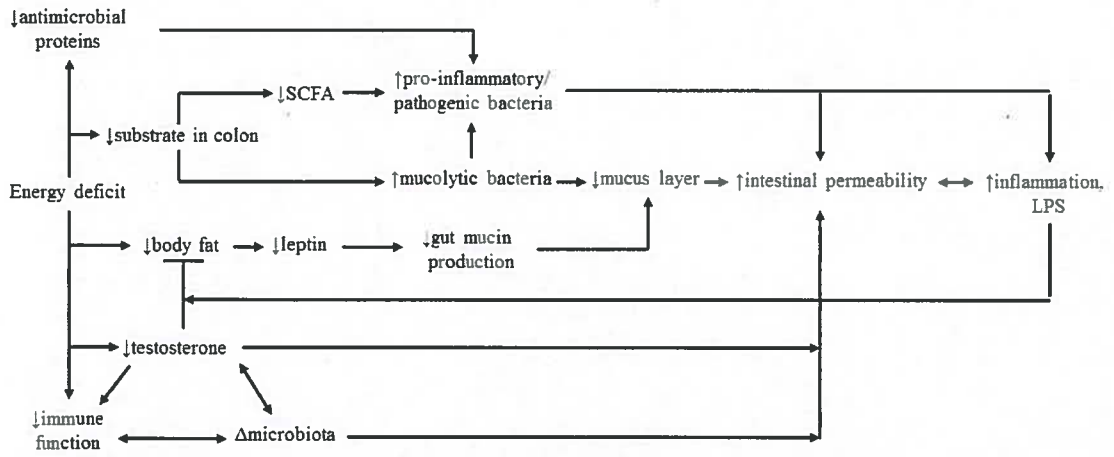
The lining of the gastrointestinal tract is both a physical and immunological barrier, acting to protect the host by deterring translocation of potentially harmful bacteria,

toxins, and antigens into systemic circulation.[74,75] The immunological barrier consists of several specialized cell types that secrete antimicrobial compounds and participate in antigen-sensing and immune response activation.[76] The physical barrier includes both a mucus layer and a layer of epithelial cells connected by protein complexes known as tight junctions. Tight junctions are particularly important to the integrity of the physical barrier, acting to regulate permeability through the opening and closing of paracellular channels through which contents of the intestinal lumen can pass.[75] Disruption or dysfunction of the gastrointestinal barrier can increase intestinal permeability, causing translocation of bacteria and their pro-inflammatory components (e.g., lipopolysaccharide [LPS], also known as endotoxin) into systemic circulation.[74] The resulting low-grade systemic inflammation increases susceptibility to acute and chronic disease,[77] and may compromise nutrient status (e.g., iron status),[78] adversely impact cognitive and physical performance,[74] and exacerbate gastrointestinal barrier dysfunction.[75]

Evidence suggests that changes in gut microbiota due to energy deficit may alter intestinal permeability (**Figure 1**). Specifically, gut bacteria capable of degrading host-derived glycans within the mucus layer outcompete other bacteria populations as energy restriction decreases the availability of undigested dietary substrates.[79] Sulfates released during degradation of mucins within the mucus layer facilitate the growth of pro-inflammatory, sulfate-reducing bacteria,[80] which can lead to decreased bacterial fermentation and short-chain fatty acid (SCFA) production. The resulting increase in colonic pH is conducive to the growth of pathogenic bacteria that suppress antimicrobial protein secretion and immune function. This combination of factors can degrade the mucus barrier, increase intestinal permeability, and subsequently affect inflammation and metabolic dysfunction. Additionally, reduced leptin secretion may alter intestinal permeability and gut microbiota composition during energy deficit as leptin stimulates gut mucin production,[81] and reduced leptin concentrations during energy deficit have been associated with changes in gut microbiota composition in mice.[82]

Emerging evidence suggests testosterone may mediate intestinal permeability. Specifically, sex hormones, including testosterone, increase expression of tight junction proteins in multiple tissues.[83,84] Testosterone is also thought to regulate immunity, with recent evidence indicating that testosterone may have a bi-directional relationship with the gut microbiome.[85] These findings suggest that testosterone maintenance could attenuate energy deficit-mediated decrements in gut microbiota composition and intestinal permeability. To our knowledge, this hypothesis has not been empirically examined. This study will address this knowledge gap by determining the effects of energy deficit and testosterone maintenance during energy deficit on the gut microbiome, intestinal permeability, and inflammation.

Figure 1. Proposed mechanisms of energy deficit-mediated effects on the gut microbiome and intestinal permeability.



Experimental Design and Overview

Physically active men (n=60) will be recruited for a 3-phase, randomized, placebo-controlled study to assess physiological and psychological responses to testosterone administration, at a dosage designed to maintain eugonadal status, during severe and sustained energy restriction. **Free-living, phase 1** will begin immediately after baseline measures are complete. Participants will be prescribed and provided individualized, 14-day eucaloric lead-in diets with 1.6 g protein·kg^{-1}·d^{-1}, 30% of total daily energy requirements from fat, and the remaining energy from carbohydrate. The 14-day lead-in diet will ensure sufficient time to acclimate to the prescribed study diet. After completing phase 1, participants will be randomly assigned to one of two, highly controlled (**live-in, phase 2**), 28-day treatment groups: energy deficit (DEF, 55% of total daily energy expenditure, TDEE) or energy deficit + testosterone maintenance (DEF+TEST). Protein intake (1.6 g protein·kg^{-1}·d^{-1}, based on day 14 total body mass) will remain constant throughout the 28-day intervention, fat will contribute 30% of total energy, and the remaining energy will be derived from carbohydrate. Exercise-induced energy expenditure (EIEE) will be increased 50% above TDEE for all participants. Energy intake will be 45% of the elevated TDEE, resulting in a 55% energy deficit (**see Table 1 for an example**).

Table 1. Example total daily energy expenditure and dietary energy and macronutrient content of the 28-d experimental diets.

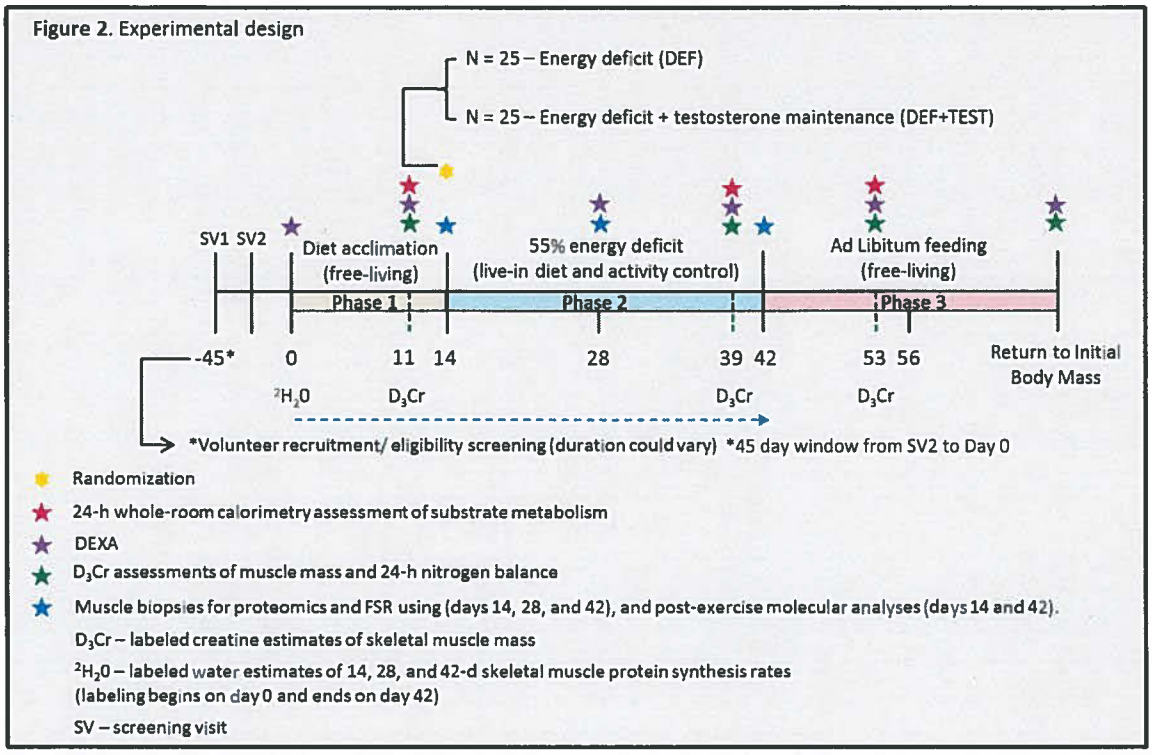
Days 0-14		**Days 15-42**	
BMR+TEF	2250	TDEE days 0-14	2500
EIEE	250	50% ↑ EIEE	1250
TDEE	2500	TDEE for all groups	3750

Energy intake, deficit, diet composition, and estimated loss of body mass during days 15-42

	DEF, DEF+TEST
TDEE	3750
Energy intake, kcal (% of TDEE)	1687 (45)
Energy deficit, kcal (% of TDEE)	2063 (55)
Protein, g (g/kg, kcal)	128 (1.6, 512)
Fat, g (% total energy, kcal)	56 (30, 506)
Carbohydrate, g (g/kg, kcal)	168 (2.1, 670)
Body mass loss, kg (% of initial body mass)	7.5 (9.4)

[1]Representative data based on a 2500 kcal TDEE for an 80 kg male for days 0-14.

Participants will be released from the metabolic center the day after completing phase 2. Participants will be allowed to return to their habitual diet and physical activity patterns to assess total body mass and, more importantly, skeletal muscle mass recovery from the intervention (**free-living, phase 3**). Recovery will be assessed after completing phase 2 to determine when body mass has been recovered within ± 2.5% of initial body mass (end of study, EOS). The duration of phase 3 will vary by participant, depending on each individual's rate of body mass regain (42-day maximum for phase 3). The experimental design is presented in **Figure 2**.



Details regarding study procedures can be found in the *Study Parameters & Procedure Descriptions* section below. See **Appendix A** Train Schedule for timing of study-specific measures.

Enrollment will begin during the spring of 2016 and we anticipate enrollment to continue for at least one year (summer 2017).

We expect that all data will be collected by September 2017. Complete data analysis, including the preparation of the final report and peer-reviewed manuscripts, will be completed in the winter of 2018.

Rationale for Duration and Severity of Energy Deficit

The duration and severity of energy deficit proposed in the current study was based on prior studies showing decrements in testosterone concentrations during energy deficit (unpublished data, Pasiakos 2015).[3,5,17,18,32] Special operations field training has been shown to decrease testosterone levels by 50-85% and total body mass by 6-16% in response to short (7 days) and longer-term (56 days) energy deficits of ≥1,000 kcal/d (unpublished, Pasiakos 2015).[3,17] A tightly controlled clinical trial that reduced energy intake by ~750 kcal/d (40%) for 21 days produced smaller reductions in testosterone (16%) and total body mass (4%).[32] Study duration, physical activity quantity and intensity, energy deficit, and dietary composition are critical factors to consider when comparing results from field and clinical studies. Therefore, in the current study, we have proposed a 28-day energy deficit that increases energy expenditure (+50%) and decreases energy intake (-55%) to levels comparable to the extreme energy deficits observed in military field studies. An energy deficit of this magnitude should produce

reductions in total body mass, lean body mass, and testosterone large enough for the intervention to be effective and create a biologically relevant and statistically significant difference between treatment groups.

Primary Study Endpoint

Total body mass will serve as the primary study endpoint and be used as a daily measure of accuracy and compliance with the diet and exercise intervention. Recovery of total body mass to pre-deficit levels (± 2.5%) or 42 days in phase 3 without recovery of body mass to pre-deficit levels will signal EOS. No other endpoint will signal EOS for any particular participant.

However, the study may be terminated if a participant withdraws, is unable to complete the prescribed exercise, has complications after undergoing the biopsy procedure, or if there is proof of noncompliance. Participants are required to complete all study measures at the given time points. In the event a procedure cannot be completed or is refused, participant continuation in the trial will be determined by the PI and MI. Noncompliance to the intervention will be addressed on a case by case basis.

Recruitment Methods

The PBRC staff has an extensive history of successfully recruiting and conducting large funded trials on exercise and dietary restriction interventions. Study recruitment will rely on previous methods, which have proven successful at enrolling volunteers who are consistent with the demographics of the region. PBRC also has a Clinical Trials Recruitment Core with 6 full-time staff dedicated solely to recruiting for clinical trials. In addition to phone calls, participants can screen via the PBRC website (https://www.pbrc.edu/clinical-trials/) and study-specific web pages are designed by the Recruiting Core. Both methods, phone and web, are funneled into a computerized participant-tracking system that allows for eligibility checks and real-time reporting. Investigators are able to track the recruitment status of their studies in real time using the PBRC intranet.

The metro Baton Rouge, LA, area has a population of 411,000. The Clinical Trials Recruitment Core receives 3,200 calls per month from potential volunteers. Study recruitment will rely on the existing methods of the Clinical Trials Recruitment Core to advertise via local media (print, radio, TV), paid targeted digital campaigns, earned media, social media (Facebook, Twitter, etc.), and collaborative relationships with local universities, sport vendors, health food vendors, etc. Targeted populations for recruitment will include PBRC employees, healthy adults (ages 18 and older), and non-military personnel.

Inclusion and Exclusion Criteria

Inclusion Criteria

- Men aged 18-39 years
- Physically active (at least 2 days per week aerobic and/or resistance exercise)

- Not taking any prescription medications and/or willing to refrain from all medication use prior to and throughout the entire study period, unless provided/approved by the study physician
- Willing to refrain from alcohol, smoking, e-cigarettes or use of any nicotine product, caffeine, and dietary supplement use throughout the entire study period.
    - At the discretion of the study physician, wash-out period for medications, supplements, and over the counter medications (OTCs) is ≥ 1-4 weeks
    - Wash-out period for caffeine and alcohol is ≥ 7 days
- Willing to live on the PBRC inpatient unit for 28 consecutive days
- Willing to have a urine drug screening
- Meets age-specific US Army body composition standards according to Army Regulation 600-9, which includes estimates of percent body fat based on height, weight, and circumference measures (neck and waist)
- Total testosterone concentration is within the normal physiological range (300-1,000 ng/dL).

Exclusion Criteria

- Musculoskeletal injuries that compromise exercise capability
- Diagnosed cardiometabolic disorders (i.e., hypertension, hyperlipidemia, kidney disease, diabetes, etc.)
- Allergies or intolerance to foods, vegetarian practices, or history of complications with lidocaine
- Anabolic steroid, human growth hormone, or nutritional testosterone precursor-like supplement use within the past 6 months
- Will not refrain from smoking (any nicotine product), alcohol, caffeine, or any other dietary supplement during the study
- Any use of antibiotics, except topical antibiotics, within 3 months of study participation
- Colonoscopy within 3 months of study participation
- Chronic use of laxatives, stool softeners, antacids, or anti-diarrheal medications (≥ once a week)
- History of gastrointestinal disease (e.g., celiac, irritable bowel syndrome, colitis, Crohn's disease)
- Restrained eater (the Three-Factor Eating Questionnaire) as assessed by the study's psychological and behavioral assessment staff
- Adults unable to consent
- Women
- Prisoners
- Metal implants, claustrophobia, head size incompatible with MRI equipment, etc.
- Sedentary or engages in <2 days of physical activity per week (aerobic and/or resistance training)
- Exceeds age-specific US Army body composition standards according to Army Regulation 600-9
- Previous history of kidney stones unless otherwise approved by the medical investigator
- Systolic blood pressure > 150 or diastolic blood pressure > 95 mmHg
- Previous history of breast or prostate cancer

- Previous history of COPD or Obstructive Sleep Apnea (OSA)
- Findings of lab results of PSA > 3ng/ml, Hematocrit > 50%, or positive urine drug screening
- Based on the investigative team's clinical judgment, a subject may not be appropriate for participation in the study.

Enrolling individuals based on US Army body composition standards (**Appendix B)** will ensure a population representative of the US military is studied.

Power analysis

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. The percentage total body mass loss (2.7 kg) attributed to lean body mass during a 21-d, higher-protein (1.6 $g \cdot kg^{-1} \cdot d^{-1}$), moderate energy deficit was approximately 30% (0.8 kg).[24] However, the proportion of total body mass loss (5.8 kg) attributed to lean body mass in response to a short-term (~7-d), military training-induced, severe energy deficit was approximately 55% (3.1 kg) (unpublished data). Based on these results, and given the proposed study will induce a 55% energy deficit for 28 days in men consuming a higher-protein diet (1.6 $g \cdot kg^{-1} \cdot d^{-1}$), lean body mass will likely account for 40% of the total body mass loss. Maintaining testosterone within normal physiological ranges is expected to attenuate the loss of lean body mass by 25%, such that those assigned to the testosterone group will lose proportionally 30%, the same percentage of lean body mass as demonstrated in our previous study. Based on these estimates, the sample size necessary to determine the estimated differences between treatments is 22 per group. However, based on previous variability in lean body mass loss in response to moderate-to-severe energy deficit, 25 participants per group is a more conservative estimate to allow for detection of treatment effects in the current study. To account for possible attrition (20%), 30 participants will be assigned to each group (60 total participants). To successfully enroll 60 participants, we request the ability to consent up to 240 participants, given only a third of those briefed and consented in past projects, with more lenient study-specific eligibility criteria, were enrolled. ***At least 50 participants will complete the intervention and enrollment will stop once 50 participants have completed the study.***

Table 2. Power Analysis and Sample Size Justification

Hypothesized Effect (mean ± SD)	
Δ Lean body mass following the 28-d intervention, kg	
DEF	3.0 ± 0.75
DEF+TEST	2.25 ± 0.75
Effect Size	1.0
Alpha	0.05
Power	0.90
Sample Size	22 per group
Expected variability	25 per group
20% study attrition	60 participants total
75% eligibility screen failure	240 total consented individuals

Enrollment will stop once 50 participants (25 per group) complete the intervention

Study Timeline

Individuals who respond to recruitment materials and indicate interest in study participation will be provided with study-specific information. If still interested, potential participants will be asked a series of demographic and health-related questions to assess eligibility via phone/web-based methods (approximately 15 min). Individuals who meet the initial inclusion/exclusion criteria via **self-reported** responses will be invited to attend two screening visits at Pennington Biomedical Research Center (PBRC). Details of each study visit are described below. See Train Schedule in **Appendix A**.

Screening Visit 1. SV1

- The study consent form will be reviewed with the participant to ensure all questions and concerns are clarified before the participant signs the consent form and before any procedures are conducted. Those who are still interested in participating will be asked to sign the consent form.
- Height, weight, blood pressure, and pulse will be measured. Participants who do not meet the height/weight criteria will have eligibility assessed by neck and waist circumference measurements to estimate percent body fat according to Army Regulation 600-9 (**Appendix B**). Circumference measurements will not be repeated during the study.
- A Physical Activity Readiness Questionnaire (PAR-Q) will be administered and CVD risk stratification assessed.
- Information regarding the participant's current medications and medical history will be recorded.
- A study dietitian will meet with the participant to discuss current eating habits and dietary requirements for each phase of the study.
- Eligibility criteria will be reviewed. All participants who maintain eligibility criteria will be scheduled for an appointment approximately 1 week later for Screening Visit 2.

Screening Visit 2. SV2

- The participant will have an EKG and a complete physical exam.
- Weight, blood pressure, pulse, and head size will be measured.
- Fasting blood will be drawn for CBC, Chem 26, PSA, testosterone, and study archives.
- Urine will be collected for a urinalysis and urine drug screening.
- A barriers interview will be conducted (**Appendix C**).
- A Three Factor Eating Questionnaire (TFEQ), or equivalent measure, will be administered to exclude restrained eaters.
- Information regarding any changes in medications or any adverse events will be recorded.
- Eligibility criteria will be reviewed. All participants who maintain eligibility criteria will be provided an accelerometer to wear, will complete a 3 day physical activity log, and will complete a 3-day food record (2 weekdays, 1 weekend day) one week prior to the start of phase 1. An appointment will be scheduled for Day 0 within 45 days of SV2.

- If more than 45 days lapse between screening measures and D0 scheduled date, participants may be asked to repeat measures to re-assess eligibility.

Phase 1: Diet Acclimation (free-living). Day 0–Day 14

- During the diet acclimation phase, participants will return to Pennington Biomedical once a day to eat and receive additional meals for the day. Participants will be weighed daily and caloric adjustments made if needed. Participants will be asked to record their activity daily throughout the phase. Additionally, 2 accelerometers (wrist- and waist-worn) will be applied at Day 0 and worn through day 14.
- Blood draws will occur on days 0, 7, and 14.
- Cognitive training will be performed on days 0, 2, 4, 6, 8, and 10 for approximately 1.5-2 hours each day. Cognitive testing will be performed on days 5 and 13 over a 2-hour period each day. The timing of training sessions is not critical.
- A DXA will be done on days 0 and 11.
- A 1-hour fMRI will be done approximately on days 5, 9, and 12.
- A food intake test with VAS and gut hormone assessments will be done on day 7.
- Participants will consume 150 mL/d $^{2}H_2O$ (i.e., heavy water) from day 0 to 7 and 100 mL/d $^{2}H_2O$ from day 8 to 14.
- Participants will be asked to provide a saliva sample on days 3, 7, and 11 to assess body water enrichment.
- Participants will ingest D_3-creatine (D_3Cr) (60 mg capsule) on day 11 for estimates of muscle mass at the end of phase 1 (urine collections on days 11, 13, 14).
- On day 11, participants will stay in the metabolic chamber and have urine nitrogen and urine creatinine collected for 24 hours.
- Participants will consume 2 g sucralose dissolved in 180 mL water on the morning of study day 11. A 24-hour urine sample collected with the nitrogen and creatinine testing will be used to analyze the sugar substitute ingestion.
- Participants will be asked to provide a stool sample on day 11 (+72 hours) for gut microbiome analysis.
- Information regarding adverse events will be collected on days 0, 7, and 14.
- A biodex familiarization test will be performed on day 0 and the actual biodex test will be performed on day 13.
- A VO_{2max} treadmill test will be performed on day 0. The test will be used to prescribe exercise intensities.
- Eating Inventories (TFEQ, FCI, FCI trait) will be completed on day 14.
- A bike familiarization ride will be performed within the first week of Phase I. The familiarization ride will be used to establish appropriate settings and exercise workloads for the experimental testing procedures.
- Muscle biopsies will be collected pre, 60 min and 360 min post a 60 min cycle ergometry exercise session on day 14.
- Time commitment during phase 1 will be about 34 hours per day on days 0-6, 8-10 and 12-13. On day 7, the time commitment will be about 4 hours. On days 11 (whole-room calorimetry day) and 14 (end of phase 1, check into PBRC metabolic ward after testing), participants will be at the facility for 24 hours.

- Participants will be randomly assigned to 1 of 2 experimental groups on day 14.

Randomization

Immediately following phase 1 testing on day 14, participants will be randomized to one of two experimental groups, either a 28-d (live-in), 55% energy deficit phase (DEF) or a 28-d (live-in), 55% energy deficit phase with exogenous testosterone administration (DEF+TEST). A randomization scheme will be determined using a block design (n=60) and age stratification (< 29 years or ≥ 29 years). Randomization will be done by a biostatistician with no direct study affiliation. Prior to the start of the study, the randomization schedule will be given to the pharmacist. The PBRC clinical research pharmacist will have no direct contact with participants. Treatment administration will be performed by a physician assistant, nurse practitioner, or nurse who will not be aware of treatment assignments. Participants and all study personnel will be blinded to treatment group. The code will be kept as a locked electronic file on a secure server by the pharmacist until study completion or there is a need to break the code for safety of the participant.

Phase 2: 55% Energy Deficit (live-in diet & activity control). Day 15–Day 42

- During the energy deficit phase, participants will live on Pennington Biomedical's inpatient unit in a 24-hour-a-day controlled setting. Once a day, participants will have their vitals (BP, pulse) measured. All meals will be eaten on the unit and monitored. Daily, participants will be weighed. Additionally, a wrist-worn accelerometer will be used throughout phase 2.
- Throughout phase 2, participants will complete supervised exercise sessions 1-4 times per day. Every 2 weeks, participants will undergo submax met carts for each mode of exercise utilized.
- Participants will consume 100 mL/d $^{2}H_{2}O$ (i.e., heavy water) from day 15 to 42.
- Participants will be asked to provide a saliva sample on days 15, 19, 23, 27, 31, 35, and 39 to assess body water enrichment.
- Blood draws will occur on days 28 and 42.
- Cognitive testing will be performed on days 15, 20, 22, 27, 29, 34, 36, 40 and 41 over a 1.5-2 hour period each day.
- A DXA scan will be done on days 28 and 39.
- A 1 hour fMRI will be done on days 36, 37, and 38.
- An injection (placebo or testosterone) will be administered on days 15, 21, 28, and 35.
- Eating Inventories (TFEQ, FCI, FCI trait) will be completed on day 42.
- Muscle biopsies will be collected at rest on day 28 and pre, 60 min and 360 min post a 60 min cycle ergometry exercise session on day 42. ***Participants will undergo 7 total muscle biopsy procedures during the entire study period (3 on one leg and 4 on the opposite leg, 3 total incisions).***
- Participants will ingest D_3-creatine (D_3Cr) (60 mg capsule) on day 39 for estimates of muscle mass at the end of phase 2 (urine collections on days 39, 41, 42).
- On day 39, participants will stay in the metabolic chamber and have urine nitrogen and urine creatinine collected for 24 hours.

- Participants will consume 2 g sucralose dissolved in 180 mL water on the morning of study day 39. A 24-hour urine sample collected for nitrogen and creatinine testing will be used to analyze the sugar substitute ingestion.
- Exercise testing for muscular strength (biodex) will be performed on day 41.
- Participants will be asked to provide a stool sample on days 25 and 39 (+72 hours) for gut microbiome analysis.
- Information regarding adverse events will be collected weekly on days 21, 28, 35, and 42.
- Participants will reside in the metabolic ward at PBRC during phase 2, thus the time commitment for this phase is 24 hours per day.
- Upon completion of each week of Phase 2, participants will receive an incentive item to aid in retention and adherence to the study protocol. Incentive items include a towel, water bottle, t-shirt, and gym bag.

Phase 3: Ab Libitum Feeding (free-living). Day 43–Return to Body Mass

- For days 43 (participants will be released after completing the final test on day 43) to EOS, participants will return to PBRC at least weekly to weigh-in.
- While participants will report to PBRC approximately once a week to weigh-in, each participant will be provided a scale to take home for daily weight measurements during this time frame. All measures will be semi-nude (t-shirt, shorts, socks) and performed after an overnight fast. Prior to distribution of the scales, they will be verified via PBRC's scale calibration verification SOP with a 50 lb. standard weight. At each weekly check, the participant will be asked to return the scale for reverification to ensure accurate weight measurements.
- Participants will complete a 3-day food record (2 weekdays, 1 weekend day) one week into phase 3 (days 50 to 56).
- Accelerometers (wrist and waist worn) will be worn on days 43 through EOS.
- A food intake test with VAS and gut hormones will be done on day 43.
- Blood draws will occur on day 56.
- Cognitive testing will be performed on day 54
- A DXA will be done on day 53.
- A 1 hour fMRI will be done on days 55, 56, and 57.
- Exercise testing for muscular strength (biodex) will be performed on day 55.
- On day 53, participants will stay in the metabolic chamber and have urine nitrogen and urine creatinine collected for 24 hours.
- Participants will consume D_3-creatine (D_3Cr) (60 mg capsule) on day 53 for estimates of muscle mass (urine collected on days 53, 55, 56).
- Time commitment during phase 3 will be about 2-3 hours per day on days 54-57. On day 43, the time commitment will be about 4 hours. On day 53 (whole-room calorimetry day), participants will be at the facility for 24 hours. On the once a week weigh-in, participants will be at the facility for approximately 30 minutes.

EOS: End of Study. EOS – EOS + 3 days

- During EOS, participants will return to Pennington Biomedical 4 consecutive days (EOS, EOS + 1, EOS +2, EOS + 3). During each visit, the participants will be weighed.

- Participants will be asked to complete a 3-d food record (2 weekdays, 1 weekend day) prior to the EOS visit. When the participant has reached approximately + 3% of initial body weight, he will be asked to begin the 3 day food record.
- Accelerometers (wrist and waist worn) will continue to be worn through EOS + 3.
- Cognitive testing, a DXA, and muscular strength (biodex) will be measured at EOS.
- A blood draw and 24 hour urine creatine and urine nitrogen will be collected at EOS.
- Participants will consume D_3-creatine (D_3Cr) (60 mg capsule) on EOS for estimates of muscle mass at the end of phase 3 (urine collected at EOS, EOS + 2, EOS + 3).
- Participants will consume 2 g sucralose dissolved in 180 mL water on the morning of EOS. A 24-hour urine sample collected for nitrogen and creatinine testing will be used to analyze the sugar substitute ingestion.
- Participants will be asked to provide a stool sample on EOS (+72 hours) for gut microbiome analysis.
- Eating Inventories (TFEQ, FCI, FCI trait) will be completed on EOS.
- A food intake test with VAS and gut hormones will be done on EOS+1.
- Information regarding any changes in medications or any adverse events will be recorded.
- Time commitment during EOS will be about 4-5 hours at EOS, 4 hours at EOS+1, and approximately 1 hour at EOS+2 and EOS+3.

PRN: As Needed Follow-up Visit

- After day 42, if the participant's testosterone levels have not returned to normal levels, a blood draw will occur every 90 days until levels have returned to within normal range (testosterone concentration ≥ 300 ng/dL).

Note: If timeline deviations are unavoidable (e.g. equipment issues, participant illness, unforeseen delays, etc.) schedule alterations, that will not affect study outcomes, may be necessary and are at the discretion of study staff.

Study Parameters & Procedure Descriptions

Objective I: Methodology

Determination of habitual dietary intake and physical activity levels

Eligible participants will complete a 3-day food diary according to instructions provided by the research team prior to the start of phase 1 (**Appendix D**). Habitual physical activity will be determined from accelerometer data obtained during the pre-study period. Physical activity patterns will be maintained at pre-study levels during phase 1. Physical activity will be verified using an accelerometer and physical activity logs during screening, phases 1 and 3; physical activity will be highly controlled and monitored during phase 2.

Determination of body composition

Height will be measured using a stadiometer. Weight will be measured (semi-nude and performed after an overnight fast) at PBRC using a calibrated digital scale at SV1 and

SV2 and daily during phase 1 and phase 2. After completing phase 2, participants will report to PBRC approximately once a week to weigh in and each participant will be provided a scale to take home for daily weight measurements (semi-nude and performed after an overnight fast).

Body composition will be determined using DXA (GE iDXA) on days 0, 11, 28, 39, 53 and EOS. Volunteers will undergo 6 DXA scans. DXA allows the non-invasive assessment of soft tissue composition by region with a precision of 1-3%.[86] Volunteers lay in the supine position on the densitometer table in shorts, t-shirts, and socks. They will be asked to remain motionless for the 5-10 min scan. These data will be used to calculate lean body mass, fat mass, bone mineral content, bone mineral density, and total body tissue mass. Calibration to external standards will be performed prior to actual data collection.

Estimates of skeletal muscle mass will be determined using the creatine (methyl-d_3) dilution method. The creatine dilution method uses basic principles of muscle creatine and creatinine biology to estimate muscle mass based on the irreversible conversion of creatine to creatinine and subsequent excretion in the urine. Participants will provide a fasting urine sample (to correct for background enrichments; **all urine samples will be second morning void before eating**) prior to ingesting a single, 60 mg dose (capsule) of D_3Cr on days 11, 39, 53, and EOS. Fasting urine samples will be collected 48 and 72 hours after each creatine pill ingestion to assess longitudinal changes in muscle mass. Research staff will provide labeled tubes for each urine collection. De-identified urine samples will be processed and frozen for future analysis by liquid chromatography–mass spectrometry (LCMS).

Determination of aerobic capacity

Aerobic capacity (i.e., peak oxygen uptake, VO_{2peak}) will be measured using an indirect open circuit respiratory system on a treadmill (day 0) during phase 1. Aerobic capacity will be used as a reference point to determine the appropriate exercise workloads necessary to meet the energy requirements for phase 2. In brief, participants will be clothed in appropriate athletic attire and perform this assessment at standard ambient indoor temperature (20-22°C) and humidity conditions (30-80%). Participants will be instructed to refrain from the consumption of food and caffeine for a minimum of 3 hours, following an overnight fast before testing, or following a light snack. Following instruction, participants will be given adequate time to become familiar with the testing procedures and allowed a 5-min self-paced warm-up on the treadmill. At the initiation of testing, the participant will put on a nose clip and a mouthpiece connected to a two way respiratory valve, which is attached to a head piece to hold it in place. Prior to beginning the running protocol, participants will walk for 5 minutes at predetermined comfortable speed and a 0% grade. The participants will then run for 4 minutes at a pace predetermined as comfortable at a 0% grade. At 4-min, the grade will be increased to 4%, followed by an additional 2% every two min thereafter until volitional exhaustion. Heart rate and ratings of perceived exertion (RPE) will be recorded during each stage. Although the testing endpoint will be volitional exhaustion, the test will be stopped immediately if the subject reports angina-like symptoms, exertional syncope, shows signs of poor perfusion (i.e., light-headedness, confusion, ataxia, pallor, cyanosis, nausea, or cold and clammy skin), or if there is a failure of testing equipment.

Testosterone and placebo administration

Participants will receive either 200 mg testosterone enanthate or placebo (sesame oil) by intramuscular injection at the conclusion of all phase 1 testing (morning of day 15) and then weekly on days 21, 28, and 35. Doses and syringes will be prepared by the PBRC clinical research pharmacy and injected by PBRC medical staff. Based on previous dose-response studies, 200 mg of testosterone enanthate was chosen as an effective dose to maintain testosterone within normal physiological ranges while minimizing risk of secondary health effects.[28,87,88]

Endocrine, hematological and physiological biomarkers

Fasted blood samples will be collected biweekly on days 0, 14, 28, 42, 56 and EOS. Blood samples will be analyzed for total testosterone, free testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), sex hormone binding globulin (SHBG), insulin-like growth factor-1 (IGF-1), estradiol, insulin, cortisol and a complete blood count with differential (CBC w/diff). In addition, blood samples will be analyzed for amino acids, lipopolysaccharide-binding protein (LBP), and a chem 15 panel on days 0, 14, 42, 56 and EOS, Prostate-specific antigen (PSA) will be assessed on days 14 and 42. Blood samples collected on days 14, 28, and 42 will be collected before the first muscle biopsy procedure. Additional blood will be collected and archived to allow for future analyses. Blood measure collection time points are shown in **Appendix E**. In addition, testosterone measurements will be taken every 90 days (starting from day 42) to assure full recovery (testosterone concentration ≥ 300 ng/dL) once the study has been completed.

Experimental physical activity

Physical activity will be controlled and supervised for accuracy starting on day 15 (i.e., start of phase 2). Varied low, moderate, and high intensity (40-85 % of predetermined VO_{2peak}) endurance-type exercise will be performed during the 28-d live-in phase to increase participants' EIEE 50% above the 14-d acclimation period TDEE. Intensity is defined as <40% of HRR for low intensity, ≥ 40% and < 60% of HRR for moderate intensity, and ≥ 60% and < 90% of HRR for high intensity. Energy expenditure will be achieved by performing at least one, but no more than four, exercise sessions per day, using a variety of endurance-type modalities (outdoor walking, treadmill [walk, run, and load carriage w/weighted vest equal to 20-35% body mass], cycle ergometer, and elliptical). **Exercise intensity will be verified biweekly and adjusted accordingly using an open circuit indirect calorimeter.**

Exercise intensity and exercise modalities will be programmed to limit the risk of developing an overuse or acute injury by alternating exercise sessions between low intensity weight-bearing modes and moderate to high intensity non-weight bearing exercise. Light calisthenics will be incorporated into the exercise regimen approximately every 3-4 days to decrease the monotony of the prescribed aerobic exercise and better simulate field operations (**Appendix F**). Standardized caloric expenditures will be determined for calisthenics exercises and integrated into individual exercise prescriptions to meet target expenditure. Calisthenics will not be done within 48 hours of testing (i.e. muscle biopsy, Biodex). Exercise will be performed daily, unless the day has been designated as a light exercise (days 21, 28, 35) or testing (days 14 and 42)

day. Light exercise days will not be void of all programed physical activity but the total increase in EIEE will be reduced by half so that participants only have to perform half the amount of exercise they perform on all other days (i.e., exercise will be walking at a low intensity). **Energy intake will be adjusted to account for the reduced level of exercise to maintain the 55% energy deficit** (e.g., for an individual with 3750 kcal/d TDEE during phase 2, 55% ED, EIEE is 1250 kcal/d and energy intake 1687 kcal/d; on light exercise days EIEE should be 625 kcal and energy intake 1062 kcal). Exercise, with the exception of the exercise prescribed for particular experimental measures (i.e., muscle biopsy studies), will not be performed on testing days 14 and 42. **Energy intake will be adjusted to account for the reduced level of exercise on test days that occur during the acclimation and 28-d intervention period to maintain body mass and the 55% energy deficit, respectively.**

Experimental diet

Energy intake will be individualized for study participants using the Mifflin St Jeor Equation with an activity factor of 1.3-1.6.[89] Diets will provide 1.6 ± 0.2 g protein·kg^{-1}·d^{-1} distributed equally across meals, 30% of total daily energy requirements from fat, with the remaining energy derived from carbohydrate.[24,26]

Registered dietitians will develop individualized 6-d menus (consisting of breakfast, lunch, dinner, snacks, and beverages) (see **Appendix G** for example menu). Meals will be prepared in advance by PBRC kitchen staff to ensure the composition of the diets is accurate. The energy content of the phase 1 diet will be sufficient to maintain body mass within ±2%. Intake will be adjusted incrementally (± 200 kcal) to achieve energy balance.

Participants will consume breakfast at the PBRC metabolic kitchen during phase 1. Participants will be weighed (see *Determination of body composition*) by research staff before breakfast is provided. Lunch, dinner, snacks, and remaining beverages (water is allowed ad libitum and not accounted for on the planned menus) will be packed and consumed offsite. Dietary compliance will be verified daily by assessing foods/beverages remaining in returned coolers and using pack-out questionnaires that allow participants to list any deviations from the provided diet (**Appendix H**). Participants will stay onsite on day 14 after testing has been completed, undergo randomization, and begin the 28-d experimental phase (phase 2) the next morning.

Energy intake will be 45% of TDEE after accounting for the 50% increase in EIEE, resulting in a 55% total daily energy deficit.

The micronutrient content of the acclimation diet will be consistent with current recommendations. Micronutrient intake during the 28-d, 55% energy deficit will likely be diminished. **To maintain operational relevance and to explore the impact of sustained, severe energy deficit on micronutrient-related markers of nutritional status, intake will not be augmented with supplementation.**

Participants will resume their self-selected, ad libitum diet during the recovery phase (phase 3). Participants will complete 3-day food records (1 weekend day, 2 weekday

days) within the week prior to day 56 and prior to EOS to be turned in to PBRC staff for monitoring and assessment.

Deuterium labeling, muscle biopsies and determination of muscle protein synthesis of individual proteins throughout the proteome

Deuterium (^2H_2O) labeling, muscle biopsies, and proteomic analyses will be used to determine protein synthesis rates for individual muscle proteins in response to the intervention. Participants will consume ^2H_2O (70%; Cambridge Isotope Laboratories, Andover MA, USA) beginning on day 0 and ending on day 42. Days 0-7 will serve as an isotopic priming phase, where participants will consume 3, 50mL doses (150 mL total) each day to achieve a target enrichment of 1-2%. During the priming phase, the first dose of each day will be consumed before breakfast and supervised by study staff to ensure compliance. The additional 2 doses will be packed out for participants to consume with subsequent meals. Participants will consume 2, 50mL doses (100 mL total) each day for the remainder of the study (days 8-42) to maintain isotopic enrichment. For days 8 through 14, participants will continue to receive one supervised dose with breakfast and the other dose at home with the dinner meal. During phase 2 (days 15-42), doses will be supervised with breakfast and dinner meals. **Body water enrichment will be determined from blood collected during the muscle biopsy days 14, 28, and 42 and saliva samples collected on days 3, 7, 11, 15, 19, 23, 27, 31, 35 and 39 (Appendix I)**.

Muscle biopsy samples of the vastus lateralis will be collected while the participants are under local anesthesia (1% lidocaine) using a 5-mm Bergstrom needle with manual suction on days 14, 28, and 42 after an overnight fast. Participants will undergo three muscle biopsy procedures (pre-exercise, 60-min and 360-min post-exercise) on days 14 (either right or left leg) and 42 (contralateral leg from day 14). The leg selected will be done via random assignment with considerations for age and treatment arm. Each of the three specimens collected on days 14 and 42 will be taken from the same incision, with the needle inserted at different angles to separate sample sites by ~5 cm. One muscle biopsy will be performed on day 28 (mid-point of the intervention). That procedure will be performed on the same leg sampled on day 14, with the incision ~7 cm apart from the incision made on day 14. Up to 250 mg (multiple passes are likely required to obtain adequate sample) of tissue will be collected with each procedure. Visible blood and connective tissue will be removed from the specimens, and samples will be frozen in liquid nitrogen before being transferred on dry ice to a -80°C freezer for storage and, ultimately, analysis. Participants will be fasted for pre-exercise and 60-min post-exercise biopsies; participants will be provided a standardized meal (25% of energy requirements) after the 60-min post-exercise biopsy.

A segment of the first (pre-exercise) muscle biopsy sample taken on days 14 and 42 and the sample collected on day 28 will be used for muscle proteomics (***detailed methods provided below***) and molecular analyses of muscle glycogen, anabolic intracellular signaling, mitochondrial biogenesis, amino acid transporter expression, androgen receptor expression, and ubiquitin-mediated proteolysis using qRT-PCR, SDS-PAGE, Western Blot, and enzymatic activity assays as previously described.[90-92] The same molecular analyses will be performed on both post-exercise specimens collected 60-min and 360-min from exercise initiation (cycle ergometry for 60-min at 2.6-

3.0 L/min) on days 14 and 42. Prior to Day 14, a cycle ergometry familiarization ride will be performed within the first week of Phase I. The familiarization ride will be used to establish appropriate settings (seat height) and exercise workloads for the experimental testing procedures. To establish experimental exercise workloads, the familiarization ride will include intermittent indirect calorimetry assessments of oxygen kinetics. The familiarization trial will be no longer than the 60 min experimental trial and stopped once the appropriate workload has been established. An absolute intensity was chosen to match the metabolic cost and total work performed and to limit the confounding effects of weight loss on relative exercise intensity. The workload will approximate 65-75% VO2peak based on an anticipated average VO2peak of 4L/min (Pasiakos PLoS One 2015, Pasiakos FASEB 2013, Pasiakos AJCN 2011).

De-identified muscle, plasma and saliva samples for proteomic and body water assessments will be frozen for future analysis.

Determination of Proteome Dynamics using Liquid chromatography-tandem mass spectrometry (LCMSMS)

Muscle samples will be thawed and homogenized for 75-sec in PBS containing 1 mM PMSF and 5 mM EDTA using a Mini-BeadBeater 8 (BioSpec, Bartlesville, OK) placed on ice for 1 min. This procedure is repeated twice and the resulting homogenate will be diluted to 10% (w/v) in PBS containing 1 mM PMSF. Protein from prepared homogenates are uniformly reduced by incubation in 10 mM DTT and SDS-PAGE sample loading buffer for 5 min at 95°C. The reduced samples are then alkylated by incubating in 15 mM iodoacetamide for 1-h at room temperature. Proteins are then fractionated by SDS-PAGE (BioRad). Using in-gel molecular weight markers, each sample will be divided into 10 molecular weight regions and subjected to overnight trypsin digestion at 37°C (Trypsin Gold, Promega, Madison, WI). Alternatively, muscle samples may be fractionated by sequential extraction of proteins into buffers containing 0.08%SDS, or 4M guanidine to yield 2 soluble fractions and 1 insoluble fraction followed by overnight in-solution digestion with trypsin at 37°C. The peptides from the resulting samples will then be extracted from the gel, dried, reconstituted in 5% acetonitrile/5% formic acid for analysis by LCMSMS.

The isotopic distributions of peptides will be measured using an Agilent 6520QToF with Chip Nano source (Agilent, Santa Clara CA). Each sample will be injected two times per analysis. Mobile phase for the LC is 3% v/v acetonitrile, 0.1% formic acid, in 18MΩ water (Buffer A) and 95% acetonitrile, 0.1% formic acid in18MΩ water (Buffer B). During the first injection, data dependent MSMS fragmentation spectra will be collected with the instrument set to collect 4 MS scans per second with up to 6 MSMS spectra from each scan. MSMS fragmentation data will be analyzed using the Agilent software package Spectrum Mill (B0.3) and protein identifications will be based on the Uniprot/Swissprot database (08/2010). The kinetic information in the isotopomer patterns will be extracted from the MS scan data using the Mass Hunter software package (B0.4) from Agilent. The peptide list with calculated neutral mass, elemental formula and retention time will be used to filter the observed isotope clusters. A visual basic application will be used to calculate peptide elemental composition from lists of peptide sequences and calculate isotopomer patterns over a range of precursor body $^{2}H_{2}O$ enrichments (p), for the

number (n) of C-H positions actively incorporating ^{2}H from body water. Fractional synthesis rates of proteins are calculated by deconvoluting the mass isotopomer pattern of newly-synthesized species as compared to unlabeled species, as described previously.[93]

Determination of fiber type (fast/slow) and cross-sectional area (CSA)
Muscle biopsy material from days 14 and 42 (pre-exercise samples) will be utilized for comparative histology, as follows: A) The proportion of muscle fiber types (type II/fast-twitch/strength and type I/slow-twitch/endurance) will be measured at day 14 (baseline) to establish subject-specific fiber type composition and again at day 42 for both treatment groups. B) CSA for each fiber type, for each subject, will be measured to identify muscle wasting based on fiber type. C) Expression changes based on cellular distribution of Smad2/3, Tak1, PGC1a, S6K will be measured to link wasting with specific cell signaling on a per fiber basis.

De-identified muscle samples for muscle phenotyping will be shipped frozen to MyoSyntax for analysis.

Determination of anabolic responsiveness/resistance
Muscle precursor cells (MPCs) from muscle biopsy segments obtained on days 14 and 42, pre and 360-min post exercise, will be used to measure cell-specific response *in vitro* to anabolic agents (*e.g.,* testosterone, DHT, Leucine) to determine whether DEF reduces anabolic responsiveness and increases anabolic resistance, and whether this can be prevented with TEST administration in the DEF+TEST group, and whether exercise in these groups can influence anabolic responsiveness/ resistance in the context of energy deficit. Participant-specific expression of PGC1a and S6K will be measured in response to anabolic exposure *in vitro* to capture cell-population based distributions of anabolic response for each subject.

De-identified muscle samples for MPCs isolation will be shipped frozen to MyoSyntax for analysis.

Determination of inflammatory profiles in blood derived cells (PBMCs) and muscle tissue derived leukocytes.
Blood and muscle derived monocyte/macrophage (e.g., M1, M2) and T cell subsets (e.g., Tregs, CD8+ T cells) will be measured to determine whether energy deficit influences inflammation in peripheral blood, in correlation with infiltration of inflammatory cell subsets and regulatory T cells into muscle, to skew muscle maintenance towards wasting. Whether catabolic/ inflammatory factors (soluble and cellular) are elevated in the DEF arm, and attenuated in the TEST+DEF arm will be determined. Cell subsets in blood and muscle will be compared with serum inflammatory factors measured as part of other sub-aims in this study proposal.

De-identified muscle and plasma samples for leukocyte and PBMC inflammatory profile analysis will be shipped frozen to MyoSyntax for analysis.

Determination of muscular strength and power

To assess the severe energy deficit, with or without testosterone treatment, muscular strength/power will be measured with isometric and isokinetic knee extension tests on days 13, 41, 55, and at EOS. A familiarization session will take place on day 0. Testing will take place prior to all daily exercise bouts. Isometric quadriceps strength, maximal power and muscular endurance will be quantified using an isokinetic dynamometer (Biodex Medical Systems, Shirley, NY). Isometric muscle strength will be measured at 75° knee flexion. This procedure will be performed three times, each separated by a 30 sec rest period. Maximal muscle strength (maximal voluntary contraction, MVC) will be determined from six maximal knee extensions at a fixed speed of 60° per second, while muscular endurance capability will be quantified during 20 repeated maximal knee extensions with movement speed fixed at 180° per second. Unilateral testing will be done on the dominant leg. The data collection form for muscular strength and power can be found in **Appendix J**.

Determination of energy expenditure, substrate oxidation and nitrogen balance.
Energy expenditure, substrate oxidation, and nitrogen balance will be measured for a 24-h period in a metabolic chamber on days 11, 39, and 53 (see **Figure 2**). The metabolic chamber stays will begin before breakfast, after completing the DXA scan, (~0800) and end before receiving breakfast the following morning (~0800).[94] Participants will receive the same meals, snacks, and beverages to control for potential differences between menu days. The timing of those meals will be similar to other days of the study, with the exception of the biopsy study days. While in the metabolic chamber, participants will perform the same amount of daily exercise typically performed during acclimation, 28-d deficit and recovery phase. For uniformity, all exercise will be performed on a cycle ergometer. Lights will be turned off at 2230, and the participants will be awakened at 0630. All urine will be collected for measurement of urinary nitrogen and creatinine excretion rates to determine nitrogen balance. Energy expenditure will be calculated by indirect calorimetry corrected for urinary nitrogen excretion and respiratory quotient.[95] Energy expenditure and substrate oxidation will be calculated for the 24-h period and also partitioned between rest, exercise and sleep. Substrate oxidation (carbohydrate, fat, and protein) will be calculated using standard equations.[96]

Objective I: Statistics

All data analyses will be based on the intention-to-treat principle using SPSS statistical software, unless otherwise noted. Data will be examined quantitatively and graphically for outliers and other artifacts that might have an undue impact on the analyses. Logarithmic or similar transformations will be applied when necessary to insure the validity of statistical procedures. All tests will be two-sided and considered statistically significant at $p < 0.05$.

Physiological outcomes
Mixed-model repeated measures ANOVA will be used to test the effects of testosterone maintenance on changes in body composition, skeletal muscle function, protein dynamics, metabolism, and biomarkers of physiological status. Mixed-models will include subject as a random factor, study day and group as fixed factors, and the day-

by-group interaction. Akaike's information criterion will be used to determine appropriate covariance structures. When a statistically significant time-by-group interaction is detected ($P < 0.05$), all possible within- and between-group comparisons will be completed, and the familywise error rate adjusted using the Bonferroni correction.

Objective II: Methodology

A comprehensive battery of questionnaires, evaluations, cognitive performance tests, personality assessment and the actigraphic assessment of daily spontaneous motor activity, sleep and circadian rhythms will be used to evaluate the effects of energy deficit and exogenous testosterone administration. Validated questionnaires will be used to examine fatigue and other aspects of mood such as depression, aggressive thoughts and perceived hunger. Risk-taking propensity and dominance will be evaluated using standardized objective procedures, and beliefs about other's feelings and intentions will be determined via a validated projective technique. Vigilance, memory and reaction time will be assessed with a standardized cognitive test battery that has established sensitivity to the impact of a wide array of real-world stressors typical of military operations.[97,98] Questionnaires, evaluations, cognitive performance tests, and personality assessments are described below and can be found in **Appendix K**.

Questionnaires/Evaluations

The **Minnesota Multiphasic Personality Inventory**[99] is one of the oldest and most widely used psychological assessment tools.[100]. Although it was originally designed to establish psychological diagnoses, the MMPI provides a broader picture of a person's basic personality characteristics. Generally, results remain stable over time; but intensive interventions have been shown to produce alterations over the course of relatively short time spans.[101] To date the MMPI-2 has not been used to study the combined impact of energy restriction and testosterone supplementation in healthy young men; however, statistically significant changes in a wide array of MMPI–2 scale scores (to include Depression and Masculinity-Femininity) were found over the course of 3 months after initiating testosterone treatment to transgender men.[102] In the present study, the MMPI-2 will be administered on days 5 and 40. The MMPI-2 takes approximately 1-2 hours to complete, depending on reading level (it is designed to require a sixth-grade reading level), and will be administered via computer software.

The **Buss–Perry Aggression Questionnaire**[103] is a 29 item questionnaire in which participants rank certain statements along a 5 point continuum from "extremely uncharacteristic of me" to "extremely characteristic of me." Examples of items include: "Once in a while I can't control the urge to strike another person", "Given enough provocation, I may hit another person", and "If somebody hits me, I hit back." The scores are normalized on a scale of 0 to 1, with 1 being the highest level of aggression. Results are shown in terms of scores on 4 scales: Physical Aggression, Verbal Aggression, Anger, and Hostility. This test has shown utility for examining the impact of testosterone on aggressive impulses,[104] and it will be administered to participants in the present study at several time points. The Buss-Perry Aggression Questionnaire takes approximately 2-3 minutes and will be administered via computer software or paper and pencil.

The **Profile of Mood States** (POMS) Questionnaire[105] is a 65-item inventory of subjective mood states that is sensitive to a wide variety of nutritional manipulations, environmental factors, sleep loss and sub-clinical doses of various drugs.[106-110] Participants rate each of 65 mood-related adjectives on a five-point scale, in response to the question, "How are you feeling right now?" The adjectives factor into six mood sub-scales (tension, depression, anger, vigor, fatigue, and confusion). The POMS will be used to assess the overall mood states of the participants in the present study. The POMS Questionnaire takes less than 5 minutes and will be administered via computer software or paper-and-pencil.

The **Satiety Labeled Intensity Magnitude** (SLIM) questionnaire is a brief questionnaire designed to assess subjective feelings of hunger and fullness. The SLIM has been shown to be a sensitive, reliable, and easy-to-use scale for measuring perceived hunger and fullness.[111] Briefly, the scale is a vertical, 100 mm, bidirectional hunger/fullness scale anchored by the terms "greatest imaginable fullness" and "greatest imaginable hunger." The participant is directed to mark the scale anywhere along the axis corresponding to their level of hunger or fullness "right now." A rating anywhere above the midpoint of the scale indicates that some degree of fullness is perceived. This scale will provide valuable information on the perceived impact of the testosterone and feeding interventions in the present study. The SLIM questionnaire takes 1 minute to complete and will be administered via paper-and-pencil or computer.

Objective Risk Assessment Tests

The **Balloon Analogue Risk Task** (BART) is a computerized test designed to measure willingness to take risks versus "play it safe." It requires the participant to fill a simulated balloon with air.[112] Points are given for maintaining the flow of air and keeping the volume of the balloon as full as possible. The more expanded the balloon gets, the more points are earned. However, all points are lost if the balloon is over-inflated and pops. The object of this task is to earn as many points as possible by keeping the balloon inflated without popping. Additionally, there is a risk-learning component to this task as some balloon colors pop with less inflation and others with more, while a third category is unpredictable. Standard administration of this task allows 30 trials. The BART takes approximately 10 minutes to complete and will be administered via computer software.

Objective Cooperation/Dominance Test

The **Ultimatum Game** is a test of negotiation and cooperation among two people. It has long been of interest in behavioral research due to the fact that results often conflict with what would be logically expected based on rational economic theory alone. In this game, two players are offered a chance to win a sum of money, and all they must do is simply divide it. The proposer suggests how to split the sum, and the responder either accepts or rejects the deal. If the deal is rejected, neither player gets anything. The rational solution, suggested by game theory, is for the proposer to offer the smallest possible share and for the responder to accept it; however, the most frequent outcome is a fair share. In fact, low proposals are often rejected by responders, and it is thought that this is due to the fact that people wish to manage their reputations (i.e., show that they will not accept being treated unfairly). Since testosterone modulates the manner in

which individuals respond aggressively to challenges, and low offers on the Ultimatum Game can be viewed as expressions of a dominance challenge, the Ultimatum Game has been used to explore aggressiveness/cooperation associated with testosterone levels. In one study,[113] it was found that men who rejected low offers had significantly higher testosterone levels than those who accepted. The Ultimatum Game takes less than 5 minutes to complete and will be administered via computer software.

Beliefs, Intentions, & Desires-of-Others Assessment

The **Reading the Mind in the Eyes Test** (RTMITE)[114] was designed to examine the ability to attribute or infer beliefs, intentions and desires of others. The test has been largely used in research addressing social cognition deficits in persons with autism, Asperger's syndrome, and schizophrenia;[115] however, it would appear useful for exploring the impact of testosterone and energy deficit in normal subjects since both of these interventions have been observed to affect mood state, and since testosterone levels have been associated with aggression, selfishness, and suspicion about the intentions of others. In the present study, participants will first be presented with an oral description of the test followed by presentation of a single image of a person's eyes and their immediate eye region, along with four descriptive words. Then they will be asked to choose the word that best describes the emotional or mental state of the person in the image. This procedure will be carried out for the 1 practice picture and then the 36 test pictures. A score of 1 will be given for each correctly chosen target word and a score of 0 will be given if a foil word is chosen. The total score thus will range from 0 to 36. The test will take approximately 10 minutes to complete and will be administered via computer software.

Cognitive/Vigilance Tests

The **Scanning Visual Vigilance Task** is a test sensitive to a wide variety of environmental conditions, nutritional factors, sleep loss, and very low doses of hypnotic drugs and stimulants.[116] The subject must continuously scan a laptop or desktop computer screen to detect the occurrence of infrequent, difficult to detect stimuli. It was designed to simulate various critical military activities that require maintenance of vigilance such as sentry duty, standing radar and sonar watch and communications monitoring. The participant must detect a faint stimulus that appears randomly on a computer screen for two seconds. Typically the stimulus will occur on average once a minute. Upon detection of the stimulus the participant will press the space bar on the keyboard as rapidly as possible. The computer will record whether or not a stimulus is detected and the response time (in milliseconds) for the detections. Responses made before or after stimulus occurrence will be recorded as false alarms. This task takes 10 minutes to complete and will be administered via computer software.

The **Psychomotor Vigilance Test** (PVT) is a test of simple visual reaction time.[117] A series of stimuli are presented at random intervals on a screen and the subject must respond as rapidly as possible when a stimulus appears. The subject hits either the left or right arrow keys to respond to the stimulus. Parameters recorded include reaction time, false alarms and number of lapses (long duration responses). PVT performance lapses refer to the times when a subject fails to respond to the task in a timely manner (i.e., > 500 msec.). The test requires subjects to sustain attention and respond to a

randomly appearing stimulus on a computer screen by pressing a button. The PVT takes 10 minutes to complete and will be administered via computer software.

The **Matching to Sample** is a test assessing short-term spatial memory (working memory) and pattern recognition skills.[107,109] The participant responds by pressing the down arrow key when the word "READY" appears on the screen. The participant is then presented with an 8 X 8 matrix of a red and green checkerboard on a color screen. The matrix is on the screen for 4 seconds. Afterwards, the sample is removed and followed by a variable delay interval during which the screen is blank (except for the word delay at the bottom of the screen). After the delay, two matrices are presented on the screen: the original sample matrix and a second matrix that differs slightly in that the color sequence of two of the squares will be reversed. The participant selects the comparison matrix by responding on the left or right arrow key that matches the original sample matrix. A response (left or right arrow key) must be made within 15 seconds; otherwise a time-out error will be recorded. Correct responses will also be recorded, as will response time to choose a matrix. The task lasts approximately 5 minutes and will be administered via computer software.

The **Grammatical Reasoning** test assesses language-based logical reasoning and has been used to assess the effects of various treatments on cognitive function.[118] It has been adapted from the Baddeley Grammatical Reasoning Test. On each trial, the letters AB or BA follows a statement. The participant must decide whether or not each statement correctly describes the order of the two letters. The "T" key on the keyboard is pressed for correct (statement is true) and the "F" key is pressed for incorrect (statement is false). Statements can be positive/negative or active/passive, and a given letter may precede/follow the other letter. A session lasts for 32 trials and is made up of the above combination of statements. The time to complete this test is approximately 5 minutes and it will be administered via computer software.

The **Borg Rating of Perceived Exertion Scale** (RPE) will be administered during exercise sessions. The RPE scale assesses self-reported perceived physical exertion. It is administered during each exercise test session immediately before and immediately after the cognitive assessment period. The scale will be presented on a video monitor and participants give a verbal response that will be recorded by an experimenter (< 1 minute).

The **N-back** task measures working memory. It requires on-line monitoring, updating, and manipulation of remembered information and allows for the parametric assessment of different working memory loads. Participants will be asked to monitor the identity or location of a series of verbal (letters) stimuli and to indicate when the currently presented stimulus is the same as the one presented "n" trials back (e.g. 0, 1, 2, or 3). For example, participants will be presented with letters one at a time in the center of the monitor and asked to determine if the letter presented is the same as a predetermined letter (0-back condition), the previous letter (1-back condition), 2 previous letters back (2-back condition) and so on. Dependent measures include response time and accuracy. This task takes approximately 15 minutes to complete and will be administered via computer software.

Sleep assessments

Actigraphic measures of sleep will be collected throughout the study using wrist-worn monitors. Actigraphy offers an easy-to-implement alternative to "gold-standard" polysomnography for measuring sleep without the need for electroencephalographic, electromyographic and electrooculographic monitoring. Actigraphy is a non-invasive method to objectively assess spontaneous motor activity, circadian rest/activity cycles and sleep using a watch-sized, wrist-worn device that uses a sensitive digital accelerometer to track the frequency of movements on a minute-by-minute basis. The information is processed through various algorithms to establish sleep/wake and sleep quality measures. The Standards of Practice Committee of the American Academy of Sleep Medicine (2007) has concluded that actigraphy provides an accurate estimate of sleep patterns in healthy people.[119]

In the present investigation, actigraphic data will be collected with the Fatigue Science ReadiBand™ actigraph or equivalent device. The wrist-worn ReadiBand™ has been validated in comparison to polysomnography (as have any alternative devices which would warrant consideration as "equivalent"), and the results have shown concordance of 90% or greater in terms of sleep-scoring accuracy. The device contains a 3-D accelerometer sampled at 16 Hz, a storage chip, and a 1.5 V battery, and it is waterproof and shock resistant. It will be worn on the dominant wrist continuously, 24-hours per day, during the acclimation (days 0 to 14), intervention (days 14 to 42), and recovery (days 42 to EOS) phases of the protocol. At the end of each week of data collection, data will be downloaded, scores for indications of sleep quantity, sleep quality, and sleep/wake timing will be calculated, and the results will be archived for subsequent analysis. Daily spontaneous motor activity will also be assessed.

Questionnaires, MMPI-2, RTMITE Test, Risk-Assessment, and Cognitive/Vigilance Tests

Training: All volunteers will undergo training and familiarization with all behavioral tests (with the exception of the MMPI-2, on which there is no "training curve"). Introductory training and familiarization on the subjective scales, RTMITE test, the risk-assessment task and Ultimatum Game, and the cognitive/vigilance assessments will occur at 6 different points during the 14-day lead-in phase of the study. The entire battery of tests (questionnaires, RTMITE, and computerized cognitive and risk tests) will be administered upon arrival at the test facility on Day 0. Subsequently, on days 2, 4, 6, 8, and 10, following the packing of take-home meals and snacks, there will be an additional test-training session (one per day) which will include all of the previously-mentioned tests with the exception of select questionnaires (i.e., those on which there is no "training curve").

Baseline Testing: On Day 13, volunteers will complete a final lead-in, pre-intervention test battery which will consist of the entire suite of tests to include the questionnaires. Data from this test session which will serve as the baseline data (i.e., before the energy deficit/testosterone phase). Providing subjects with 6 pre-baseline test-training sessions along with 1 baseline questionnaire-familiarization session should be more than sufficient to minimize the influence of training effects on all of these instruments,

and therefore should ensure valid baseline results from the Day-13, pre-energy-deficit/testosterone-intervention phase of the protocol.

28-d Energy deficit testing: Once the live-in, intervention portion of the protocol begins, volunteers will be asked to complete all of the subjective questionnaires (excluding the MMPI—see below), and risk/cognitive tests at 8 different points prior to the time at which they will return to free living. These cognitive test sessions will occur on Days 15, 20, 22, 27, 29, 34, 36, and 41. Note that 3 pairs of these sessions (D20 and D22, D27 and D29, and D34 and D36) are positioned so that they immediately surround the second, third, and fourth testosterone (or placebo) administrations on days 21, 28, and 35 in order to facilitate the examination of acute pre/post dose effects. Session on D15 occurs on the same day as the first injection. A determination regarding which sets of sessions will most appropriately describe the overall cumulative impact of the testosterone/calorie-deprivation intervention will depend on whether or not a preliminary analysis indicates the presence of significant differences between the 3 pairs of pre/post testosterone-administration sessions. Regardless of the outcome of this analysis, the session on Day 41 which is 1 day prior to the end of the entire intervention cycle (and 6 days after the final testosterone dose administration) will provide a valid end-point assessment of any cumulative calorie-deprivation/testosterone effects. With regard to the MMPI-2, it will be administered only on a single pre-intervention day (Day 5) and a single near-end-of-intervention day (Day 40) to explore the impact of caloric restriction and testosterone supplementation on several of the more stable aspects of personality.

Recovery/Ad-libitum feeding testing: During the final portion of the protocol, volunteers will be asked to complete two final questionnaire/test assessment sessions. The first will be on Day 54, and the second will occur at EOS.

Functional MRI assessments

Participants will perform a total of six tasks during acquisition of functional MRI (fMRI) data within the MRI machine. Each task will be performed during phases 1, 2, and 3. Tasks 1 and 2 will be performed on days 5, 36, and 55; tasks 3 and 4 will be performed on days 9, 37, and 56; and tasks 5 and 6 will be performed on days 12, 38, and 57. The duration of each task is expected to be about 20 minutes. With 40 minutes of task performance, 5-10 minutes of structural MRI scanning performed to facilitate fMRI data analysis, and 5-10 minutes transitioning into and out of the MRI scanner, each MRI session is expected to take approximately 1 hour. For timing purposes, task coupling (ex: 1 and 2 varied to 1 and 3) may be adjusted to maintain approximately 1 hour of MRI total time. Additionally, scans in phase 1 may be completed on different train schedule days (Day 1 through Day 13) in the phase to aid in scheduling.

General MRI methodology: At the time of recruitment, each participant will first fill out a detailed screening form indicating all possible contraindications to MR scanning. Study personnel will review the form and clarify any uncertainties with the participant. On the day of scanning, participants will be asked to sit in front of a laptop computer outside the MRI scanner and perform practice versions of the tasks they are scheduled to perform in the scanner that day. Then, the MR technician will re-screen the participant for MRI contraindications out of caution. The MR technician

will then insure that all metallic items are removed from the exterior of the body of the participant, and that clothing that might cause image artifacts are removed. Participants wearing simple cotton clothing are expected to be able to remain clothed although those with suspected metallic materials in upper body clothing may be required to change into a hospital gown. Headphones are placed over the ears to remove MRI scanner noise and deliver audio stimuli during the task. The MR technician will then place the participant on the MR table, supplying cushioning under the neck and legs as needed to insure comfort. A blanket will be provided to participants that feel cold on the MR table. A belt-like respiratory monitor will be affixed around the waist and a pulse oximeter will be clipped onto a finger or toe for physiological monitoring. The MR coil will then be placed over the head, button response boxes are placed in the hands, and the participant is then inserted into the MRI tube by the MR technician. Inside the MRI tube, the participant listens to audio stimuli, views images and words projected onto a screen, and initiates task responses by clicking buttons with their fingers.

T**ask 1: Risk taking propensity:** The life-cash version of The Gambling Task assesses the propensity for high-risk, high-reward decisions, with differing reward domains (life and cash respectively) [120]. In repeated trials of a simple game, participants are asked to click buttons to choose between a high-risk, high-reward outcome; and a low-risk, low-reward outcome. The task will determine whether the testosterone intervention increases the tendency to make risky decisions in the context of prolonged energy deficit.

Task 2: Provoked aggression: In this task, the participant will be fitted with an MR-safe device designed to apply a electrical stimulus to an application site on the arm (STM100C, BioPac Systems, Inc). The player engages in a simple game with a digital opponent; after each round of the game, the winner of the round is allowed to apply the stimulus to the opponent, and is instructed to set the intensity of the applied pain (modulated by the number of electrical stimuli applied to the arm) to a value of their choosing. The participant is not told that there is no real human opponent, and both the outcomes of the trials and the intensities of the applied electrical stimulus are actually preordained. These task parameters are set in such a way as to test specific hypotheses about the participant's propensity toward retaliatory increases in applied pain level. The task gives a sense of the degree to which prolonged energy deficit and the testosterone intervention heighten the propensity toward retaliatory aggression against a provoking adversary, across varying levels of provocation [121].

T**ask 3: Multiple Source Interference Task (MSIT):** In the multi-source interference task (MSIT), each trial shows the participant a string of three numbers including digits 1 to 3, and requires the participant to use a 3-button response box to identify which of the three numbers appears only once in the string. There are two sources of interference: the two other distracting numbers in the string, and positioning of the probe number that is discordant with the position of the corresponding button on the response box [122]. Besides providing an indication of inhibitory control function, this may be the most robustly validated fMRI task in existence, with extensive data showing similar patterns of activation across individuals and across MRI scanners.

Task 4: Working memory (AX CPT): The AX continuous performance task[123] requires individuals to click a response button when they observe an X that has been followed by an A in a stream of characters displayed on the screen. Performance on the task reflects the ability to maintain a shifting buffer of recently seen characters. The CNTRCS consortium selected this task as the test of working memory that is most well characterized and ready for translation into clinical trials in neuropsychiatric disorders. The AXCPT also has a robust and growing fMRI literature.

Task 5: Attention Network Task (ANT): The Attention Network Task (ANT) probes multiple aspects of attentional and inhibitory control. Participants are presented with a line of arrows, and is required to click a lefthand or righthand button depending on whether the center arrow points to the left or right respectively. The participant is required to suppress multiple distracting spatial cues to provide this correct response. It is very well characterized from a large number of behavioral studies and has several fMRI studies as well. It provides an assessment of spatial attention and how it is modified by the presence of distracting information.

Task 6: Emotional reactivity: In this task, participants are presented with a series of trials in which a probe image of a human face shown in the center of the screen must be identified to a face shown either on the left or right side of the screen. The facial expression shown in the probe image has differing emotional valence (*i.e.,* angry or neutral). Emotional face matching provides an assessment of brain reactivity to emotional stimuli even when the task does not require overt emotion processing [124]. These tasks have consistently shown greater brain activity due to emotionally valent image content in normal individuals and enhancement of this effect in individuals who underwent acute testosterone supplementation. The task will assess whether the effects of testosterone on covert emotional processing is enhanced by prolonged energy deficit.

Objective II: Statistics

Individualized scale scores from each of the questionnaires, the MMPI-2, and the RTMITE, the scores from the Ultimatum Game, the number of points earned on the Balloon Task, and dependent measures (such as accuracy scores and reaction times) on the vigilance, memory, and reasoning tasks will be analyzed with a series of mixed-factorial ANOVAs. Significant main effects and interactions will be pursued with Analysis of Simple Effects followed by appropriate linear contrasts and pairwise comparisons.

On all assessments there will be 2 levels of the grouping factor: *Intervention* (Energy Deficit-Androgen Maintenance and Energy Deficit+Androgen Maintenance). For preliminary examination of immediate pre/post-dose effects (on tests other than MMPI), the primary analysis will include the single grouping factor of *Intervention* with two within-subjects factors: *Dose-iteration* (Dose2, Dose3, Dose4) and *Pre-post* (Pre-dose, Post-dose). For examination of cumulative treatment effects (on tests other than MMPI), the primary analyses will include the *Intervention* grouping factor (Energy

Deficit-Androgen Maintenance and Energy Deficit+Androgen Maintenance) and a single within-subjects factor labeled *Session*, minimally with 7 levels (Baseline, Intervention1, Intervention2, Intervention3, Intervention4, Recovery1, and Recovery2). The specific sessions chosen for this "cumulative-treatment-effect" analysis will depend on whether or not acute testosterone dose effects are found in the previously-described separate examination of immediate pre/post dose effects (described above). If acute post-dose effects appear to have occurred, the *Sessions* will consist of: Baseline-Day13, Live-In-Day21, Live-In-Day27, Live-In-Day34, Live-In-Day41, AdLib-Day54, and AdLib-Day83. In this case, note that all of the Live-In sessions are 6 days removed from (i.e., following) the testosterone dose administrations. If the preliminary analysis indicates no differences between the immediate pre/post dose administrations, an analysis may be performed on all of the data-collection sessions, or possibly only on the Live-In sessions that immediately follow each of the testosterone dose administrations (i.e., Baseline-Day13, Live-In-Day15, Live-In-Day22, Live-In-Day29, Live-In-Day36, AdLib-Day54, and AdLib-Day83. In this latter case, note that all of the Live-In sessions occur on the very next day after testosterone has been injected. With regard to the MMPI-2, since it is only to be administered twice, there will be 2 levels of the *Intervention* grouping factor and 2 levels of the *Session* within-subjects factor (Baseline, and End-Of-Intervention Day 40).

ReadiBand activity/sleep-monitoring data (sleep duration, sleep latency, and sleep efficiency) will be analyzed via a mixed factorial ANOVA for *Intervention* (Energy Deficit-Androgen Maintenance and Energy Deficit+Androgen Maintenance) by *Period* (Baseline, Live-In1, Live-In2, Live-In3, Live-In4, Live-In5, Ad-lib). In addition, average sleep data during the acclimation (free-living) phase will be more generally compared to average sleep data during the intervention and ad-libitum (free-living) phases with a 2x3 ANOVA. Significant interactions from both sets of analyses will be followed up with Analysis of Simple effects and appropriate post-hoc comparisons. Depending on the pattern of observed effects, a more fine-grained analysis (which would potentially include every night of ReadiBand data) may be conducted as well.

Polymorphism assays for genes related to behavior will be conducted using nucleotides extracted from whole blood collected at the initial or a subsequent blood sample (~ 1 mL). A series of polymorphisms associated with differences in the stress response, testosterone-related behavioral traits such as cooperation, completion and aggression, cognitive and endocrine function as well as skeletal muscle adaptive responses to stress will be assessed and may include (but not be limited to): OXTR, AVPR1a (social and emotional regulation) CRHR1 and FKBP5 (HPA axis reactivity); COMT, 5-HTTLPR, DRD2, GABRA6, TCAT (neurotransmitters); and BDNF (synaptic plasticity).[62-64] All polymorphism assays analyses will be performed on a fee-for-service basis at the Lincoln Laboratory at MIT; all samples will be coded using participant number.

fMRI data will be managed locally by Pennington Biomedical's Imaging Core. Across the 6 tasks, fMRI data will be analyzed in conjunction with investigators/consultants at USARIEM. Data will be analyzed for *Intervention* (Energy Deficit-Androgen Maintenance and Energy Deficit+Androgen Maintenance) by *Period* (Baseline, Live-In1, Live-In2, Live-In3, Live-In4, Live-In5, Ad-lib) effects.

Objective III A-B: Methodology

Determination of appetite and endocrine mediators of appetite
Assessment of appetite and endocrine mediators of appetite will be completed after 1 week of diet acclimation (day 7, baseline), on day 43 (post-energy deficit), and upon return to initial body mass (EOS+1). Participants will receive a fixed-portion breakfast meal (0 min) and an ad libitum lunch meal (180 min). Subjectively rated appetite and blood samples will be collected before and periodically following each meal (**Table 3 and Table 4**).

Table 3. Baseline (day 7) and recovery (EOS+1) appetite experiment timeline.

Time (min)	-15	0	30	60	120	180	185	Post-meal
Meal		x					x	
VAS	x		x	x	x	x		X
Bloods	x		x	x	x	x		

Table 4. Post-energy deficit appetite experiments (day 43).

Time (min)	-15	0	30	60	120	180	185	215	245	305	365
Meal		x					x				
VAS	x		x	x	x	x		X	x	x	x
Bloods	x		x	x	x	x		X	x	x	x

VAS, visual analog scale.

A fixed-portion test meal will be served as a breakfast meal on the morning of study days 7, 43 and at EOS+1. This meal will have mixed macronutrient content and be prepared according to a standard procedure. For each participant the energy content of the meal will be equivalent to 20% of the TDEE prescribed for that individual on study days 0-14. Water in the amount of 240 g will be provided during the meal. Participants will be instructed to consume all of the water before completing the meal and will not be permitted additional water during the meal.

A second meal, the lunch meal, will be consumed *ad libitum* 180 min following provision of the breakfast meal. The lunch meal will have a mixed macronutrient content, consist of a single item (e.g., lasagna), and be prepared according to a standard procedure by research staff. A portion calorically equivalent to at least 75% of the TDEE prescribed for that individual on study days 0-14 will be served to ensure that food intake is not limited by the amount served. Participants will be instructed to eat until comfortably full, and be permitted to eat as much or as little as desired. There will be no restrictions on meal duration. The amount of uneaten food will be weighed and the energy content of the portion consumed calculated. Water in the amount of 240 g will be provided. Participants will be instructed to consume all of the water before completing the meal and will not be permitted additional water during the meal. (**Diets will be adjusted on day 7 to account for the appetite assessment**)

In the intervals between meals participants will be required to drink 360 mL water, and will not be permitted access to any additional food or beverage.

At each blood draw, self-reported fullness, hunger, prospective consumption, and desire to eat will be measured using 100-mm visual analog scales (**Appendix K**).[125] The visual analog scales will be administered by paper and pencil or may be administered on a computer.

Biomarkers of appetite regulation.
An indwelling intravenous catheter into the antecubital vein or forearm of the participant's arm will be placed 30 min prior to the breakfast meal. A fasted blood sample will be taken 15 min before beginning the meal. Blood will be sampled according to the timelines in T**ables 3 and 4**. At each time point 14.5 mL blood will be collected for a total of 275.5 mL over the full study to measure endocrine mediators of appetite. If at any point during the testing period the catheter becomes clogged or is no longer patent it will be removed and replaced. Serum will be analyzed for leptin and insulin (measured at -15 min only),serum for glucose,and plasma for acyl ghrelin and des-acyl ghrelin at PBRC using commercially available assays. Additional samples will be collected at each timepont and archived for future tests. All samples will be collected using appropriate preservatives to minimize degradation. Blood measure collection time points are shown in **Appendix E**.

Determination of gut microbiome composition and activity, and intestinal permeability
A single fecal sample will be collected at 4 time points (end of run-in diet [days 11-14], mid-point [days 25-28] and end of intervention [days 39-42], and upon return to initial body weight [EOS-EOS+3]) during the study to assess gut microbiota composition and function. At each time point participants will be given a ≤72-h window to collect a usable sample. A usable sample is defined as being > 25g wet weight, and having been delivered to study staff within 12-h of defecation while being kept cold but not frozen from the time of collection to delivery. If a participant does not provide a usable sample within the timeframe noted above, the collection period will be extended until a usable sample is produced. To collect fecal samples, all participants will be given pre-labeled containers with covers and a plastic device to "hold" the container in the toilet. Participants will defecate into the collection container which will then be given to study staff. During free-living phases of the study participants will be given plastic sealable bags, a cooler and ice packs to store and transport the samples from home (**Appendix L**).

Samples will be processed as soon as possible and within 12-h of defecation. Aliquots for 16s rRNA gene sequencing, transcriptomics, metabolomics, short-chain fatty acids (SCFA) analysis and archiving will be frozen at -80°C. DNA and RNA will be extracted from samples using commercially available kits. Microbiota composition will be assessed by sequencing amplicons targeting the V4 region of the bacterial 16S rRNA gene using appropriate primers and unique barcodes for each sample.[126] Transcriptomics will be completed using RNA-seq. Sequencing will be completed on the Illumina MiSeq or a similar platform, [127] and completed at the Broad Institute (Cambridge, MA) or by a similar entity on a fee-for-service basis. As gut microbiota composition analysis is a rapidly evolving field, the selection of primers, the 16S rRNA

gene variable region to sequence, and the sequencing platform may be modified to be consistent with the literature and technology available at the time of analysis. Quality control of reads and taxonomic assignment (phyla to operational taxonomic unit-level) will be completed using established software and databases.[128] Metabolomics will be completed by Metabolon (Durham, North Carolina) or by a similar entity on a fee-for-service basis. SCFA will be measured as markers of bacterial fermentation at the US Army Natick Soldier Research Development and Engineering Center (NSRDEC) or elsewhere on a fee-for-service basis using gas chromatography and a flame-ionization detector [129] or an equivalent method.

Urine collection and assessment of gastrointestinal permeability
Differential sugar absorption tests will be used to provide a functional assessment of gastrointestinal permeability. [130] For this test participants will consume 2 g sucralose dissolved in 180 mL water on the morning of study days 11, 39, and upon return to initial body mass (EOS). Sucralose is a sugar substitute commonly used as a sweetener in a variety of food products. Consumption of the solution will be conducted under staff supervision. Participants will then collect a urine sample 24-h after sugar substitute ingestion. Aliquots will be taken and frozen immediately. The sucralose concentration will be analyzed by PBRC. The sucralose ratio in the 24hr urine collection will provide measures of whole gut permeability. [130] Prior to testing days, participants will undergo a 2-day washout period of sucralose- containing beverages and foods.

Determination of eating attitudes and behaviors
Several questionnaires will be administered with participants in a fasted state at SV2 and on study days 14, 42 and EOS to determine the effects of testosterone maintenance during energy deficit on eating behaviors and food cravings. We will also explore associations between these outcomes and gut microbiota composition. The Three Factor Eating Questionnaire (TFEQ) will be used to measure hunger, dietary restraint and disinhibition.[131] Food cravings will be assessed using the Food Cravings Questionnaire-trait (FCQ-trait) and the Food Cravings Inventory 2 (FCI-2) which measures the frequency of cravings for specific types of foods. The TFEQ and FCQ-trait are publicly available. The FCI will be purchased from PBRC or used with permission. The TFEQ takes about 10 minutes, the FCQ-trait 5 minutes, and the FCI-2 5 minutes to complete and will be administered via the REDCap system.

Objective III A-B: Statistics

Data will be examined quantitatively and graphically for outliers and other artifacts that might have an undue impact on the analyses. Logarithmic or similar transformations will be applied when necessary to insure the validity of statistical procedures. All tests will be two-sided and considered statistically significant at $P < 0.05$. Data analysis will be completed using SPSS statistical software unless otherwise noted.

Physiological outcomes
Mixed-model repeated measures ANOVA will be used to test the effects of testosterone maintenance on changes in appetite, postprandial endocrine responses, intestinal permeability, and SCFA concentrations over time. Postprandial appetite ratings and responses of endocrine mediators of appetite will be summarized using area under the

curve and peak/nadir concentration prior to analysis. Mixed-models will include subject as a random factor, study day and group as fixed factors, and the day-by-group interaction. Akaike's information criterion will be used to determine appropriate covariance structures. When a statistically significant time-by-group interaction is detected ($P < 0.05$), all possible within- and between-group comparisons will be completed, and the familywise error rate adjusted using the Bonferroni correction.

Psychological outcomes

Questionnaires will be scored according to questionnaire-specific procedures. Scores will then be analyzed by mixed-model repeated measures ANOVA. Mixed-models will include subject as a random factor, test session or study day and group as fixed factors, and the session-by-group or day-by-group interaction. Akaike's information criterion will be used to determine appropriate covariance structures. When a statistically significant time-by-group interaction is detected ($P < 0.05$), all possible within- and between-group comparisons will be completed, and the familywise error rate adjusted using the Bonferroni correction.

Gut microbiota composition and metabolomics

Metabolomics data will be visualized using hierarchical average-linkage clustering and principal components analysis. Taxonomic data will be visualized using hierarchical average-linkage clustering and principal coordinates analysis of beta (i.e., between samples) diversity scores (e.g., Bray-Curtis, and weighted and unweighted UniFrac). Alpha (i.e., within-sample) diversity will be calculated for taxonomic data using Shannon, Simpson and Chao1 indices. Mixed-model repeated measures ANOVA will be used to test the effects of testosterone maintenance on changes in metabolite concentrations, alpha diversity, and the relative abundance of individual taxa over time. Models will include study day and group as fixed factors, and their interaction. The Benjamini-Hochberg correction will be used to control the false discovery rate for main effects of time, group and the time-by-group interactions resulting from taxa-specific models. When a statistically significant time-by-group interaction is detected ($Q < 0.05$), post hoc within- and between-group comparisons will be completed and the familywise error rate adjusted using the Bonferroni correction. Linear discriminant analysis will be used to identify between-group differences in metabolite concentrations and relative abundances of taxa at each time point. Data analysis will be completed using SPSS, XLSTAT, R, Qiime or similar software as needed.

Relationships between gut microbiota composition, metabolites, and physiological and psychological outcomes

Changes in gut microbiota composition, metabolites, and physiological and psychological outcomes during DEF and ad libitum feeding will be examined separately by Pearson's correlation or Spearman's rank correlation as appropriate. False discovery rate will be controlled using the Benjamini-Hochberg correction. Procrustes analysis will be used to examine the relationship between gut microbiota composition and metabolite profiles. Redundancy analysis will be used to examine the amount of variance in physiological and psychological outcomes explained by gut microbiota composition and metabolite profiles. Data analysis will be completed using XLSTAT, R or similar software as needed.

Data and Specimen Management

Study participants will be assigned unique subject identification (ID) numbers. Study subject ID numbers will be used on all data collection instruments, to include questionnaires, data collection forms, biological specimen tubes, and computer records. A master list linking the participants' names and ID numbers will be kept in a password-protected computer file with access restricted to the PI and study navigator. Biological samples that are moved off-site (including to USARIEM) for analysis will not contain any personally identifiable information and will be labeled with only the unique subject ID numbers. Staff at these sites will not have access to the master list at any time.

Data collection forms will be kept under lock and key, or password-protected if computerized, and under the control of the PI, associate PIs, and project coordinator. Only personnel assigned to the research study by the PI will have access to the data. Hard-copy data records will be stored for a minimum of 3 years and a maximum of 5 years from the time the study is completed and then destroyed.
The PBRC has a fully integrated, campuswide, automated data management system. All data are entered into a Central Database using existing methodology that has been fully validated and undergoes continuous quality assurance by the PBRC Research Computing Core and NORC. Most data are automatically uploaded from the instruments that measure the endpoint. All self-report inventories and questionnaires will be completed in REDCap via surveys. Participants will be asked to complete the survey via laptop, computer, or tablet. Data will be exported from REDCap for analysis. Exercise testing data will be downloaded from the Parvo Medics' TrueOne® 2400 cart directly following each test and reviewed for integrity. All data are backed up daily, and the Research Computing Core at the PBRC oversees all data management.

Blood, muscle, and urine samples will be stored frozen at PBRC until analysis can be completed. Specific muscle, saliva, and blood samples for proteomic and body water assessments will be frozen for future analysis. Specific muscle and blood samples will be shipped to MyoSyntax for further analysis. Fecal samples will be initially processed at PBRC and stored frozen until analysis. Fecal samples or bacterial DNA extracted from fecal samples will be frozen and shipped to NSRDEC and the Broad Institute (Cambridge, MA) for analysis. Packaging and shipping of biological samples will be overseen by the PI, associate PIs, or study navigator, and will be completed in accordance with International Air Transport Association regulations to ensure that viable biological samples reach their intended destination.

Any blood, muscle, urine, or fecal samples remaining after analysis will be stored indefinitely to assess biomarkers associated with the study outcomes. This includes bacterial RNA analysis in collected fecal samples, metabolomics analysis in archived fecal/plasma/serum samples, and any biomarkers of nutritional status not currently identified in the protocol. Any use of the samples outside of this defined protocol will be submitted as a protocol amendment or a new protocol.

Standardization of Procedures and Quality Control

The research team has extensive experience using the procedures and methods required to conduct this study. Standard operating procedures in place throughout the units at Pennington Biomedical will be utilized for repeatable, valid data collection and

quality.

Data Analyses

See specific statistic sections listed under each objective.

Provisions to Monitor the Data to Ensure the Safety of Subjects

The PI and/or IRB of record will appoint a Research Monitor or monitors to ensure that voluntary participation is clearly and adequately stressed during the recruitment process. The monitor may be a member of the DSMB. The appointed DSMB member to serve as the Research Monitor is Dr. Timothy Church. This appointee will also ensure that the information provided about the research is clear, adequate and accurate. He can be called upon to interview human subjects, consult with others outside of the study or with the investigators. He will have the responsibility to promptly report his observations and finding to the IRB or other designated official. The research monitor has the authority to stop the research protocol in progress, remove an individual subject from the protocol and take whatever steps are necessary to protect the safety and well-being of human subject until the IRB can assess the monitor's report.

Data and Safety Monitoring Board

This study will use a data and safety monitoring board (DSMB). The DSMB will receive quarterly reports via email. One or more meetings each year may be conducted in person or via conference call if deemed appropriate by the DSMB chair. Prior to the start of recruitment the DSMB will give formal approval of the study protocol and informed consent.

- Size and Composition: The DSMB will consist of 4 members both internal and external to the Pennington Biomedical Research Center. The planned composition is as follows: Biostatistician (1), Exercise Physiologist (1), Clinician (1), Layperson (1)
- Major Responsibilities of Members:
  -Sign and abide by a statement of confidentiality
  -Disclose any actual or potential conflicts of interest
  -Oversee safety of participants to include review of adverse events
  -Review reports of related studies as appropriate
  -Review major proposed modifications
  -Monitor recruitment and adherence
- Reports: Following each meeting, the DSMB will provide written documentation regarding findings for the study as a whole and any relevant recommendations related to continuing, changing, or terminating the study. All DSMB recommendations will be submitted to the Principal Investigator and/or his designee, with a copy provided to the Pennington Biomedical Research Center IRB. Annually, the DMSB chair will provide a written summary report approving that the study can continue.
- Qualifications and Responsibilities of the Safety Officer: The Safety Officer for this trial will be familiar with the adverse event definitions and reporting requirements for the study. The Safety Officer will review reports sent by the study coordinator

as they occur and will determine whether there is any corrective action or stopping rule violation. The safety officer will send written documentation of the decision to the PI.

Adverse Events

Serious adverse events are defined to include:

- Death
- A life-threatening event
- Severe illness including worsening of a pre-existing condition, injury or accidents
- An inpatient hospitalization, surgical procedure, or a treatment to prevent a SAE
- A permanent disability or incapacity
- A clinically significant abnormal laboratory or diagnostic test result
- Any other event that, in opinion of the principal investigator or study physician, might have resulted in a serious adverse event if medical intervention had not been initiated

For this trial's purposes, an **adverse event or experience** is defined as any health-related unfavorable or unintended medical occurrence that happens after randomization. Examples of Adverse Events include but are not limited to the following:

- A clinically significant laboratory or clinical test result at follow up assessments
- An event that results in 3 consecutive missed exercise sessions
- An event that requires a visit to a physician because it alters participant's ability to do physical activity
- An event that occurs as a result of a study procedure which is not listed in the Risks section of the consent

Adverse events will be reported to the study PI, study coordinator, Chair of IRB, Chair of the study DSMB, and Safety Officer throughout the trial as necessary. Adverse event data will be collected from Baseline (Day 0) until the final closeout visit. Adverse Events classified as serious will be reported from the date of consent through the final closeout visit. Adverse Event Data will be analyzed quarterly, but serious or life-threatening adverse events require immediate reporting and follow-up. We anticipate most adverse events will be mild and the participant will be able to resume intervention activities within a day or two of reporting the event.

In the event an adverse event occurs on campus results in a serious or life threatening situation, the investigator or other project staff present will begin emergency measures, as appropriate, and call 911.

- For minor physical injury, the individual will be encouraged to see a health care practitioner of his or her choice.
- If the study participant experiences psychological or emotional distress, the project staff will cease research activities and attempt to calm and reassure the participant. The participant will be directed to an appropriate health care practitioner for further assessment and treatment as needed.

- The investigator and/or project staff will record detailed narrative notes describing the adverse event they witnessed or that was reported by participant. The Medical Safety Officer will complete the form Notification of an Adverse Event.

Adverse Event reporting will follow the requirements of the IRB of the Pennington Biomedical Research Center. Serious adverse events that are unexpected and related to the study will be reported within 48 hours. Other adverse events that are not serious but are unexpected and are associated with the study procedures will be reported within 10 days.

Safety Measures During Physical Activity

Exercise interventions are conducted on-site and all sessions are conducted and supervised by **trained PBRC exercise interventionists,** who monitor potential adverse experiences and symptoms. During the physical activity sessions a defibrillator and on-site trained staff are available to deal with medical emergencies. Also, institutional and community EMS services are activated if needed. Participants will be taught the importance and proper method of **warming-up** prior to and **cooling-down** following structured activity sessions. Heart rate will be monitored throughout the intervention sessions. If at any point during a physical activity session, participants develop chest pain, shortness of breath, or dizziness, they are instructed to rest and to contact their physicians if these symptoms persist or recur with further physical activity.

Procedures to minimize discomfort include **warm-up and cool-down activities** that include stretching, light walking or cycling. Participants are also **supervised and instructed** on correct physical activity techniques.

If for any reason the participant reports an injury, chest pain, shortness of breath, or dizziness, they are referred to their doctor, or the study clinician calls the doctor or other health care provider. In addition, specific criteria for **suspending or stopping physical activity** are developed to adjust the program for **intercurrent illness**.

Stopping Rules

There is more than minimal risk for participating in this trial. Nevertheless, in addition to monitoring recruitment and compliance to the intervention, we also will monitor the rates of injury in our participants. The safety officers, in conjunction with the study investigators, will alert the IRB and DSMB if a larger than reasonably expected injury rate occurs in the treatment groups. Other issues that are related to the stopping rules include:

- New information – It is unlikely that new information will become available during this study that would result in discontinuing the trial.
- Limits of assumption – It is possible that the value of data analysis will be limited by differences between the intervention groups at baseline or because of study dropouts or missing data. Baseline differences will be analyzed annually and effects on the power to detect differences in the outcome measures will be evaluated and discussed with the PI, safety officer, and the USARIEM Project Officer. Although an excessive number

of dropouts could occur, this has not been our past experience. In similar studies completed by USARIEM, the dropout rate was approximately 10%. If the dropout rate for the proposed study exceeds 20%, the safety officer will initiate a meeting with the PI to discuss strategies to increase retention. If the dropout rate exceeds 50%, the safety officer will meet with the study investigators to determine whether or not the study should continue.

- Limit of rules – We acknowledge that circumstances, other than what are listed, may justify stopping the study.

Withdrawal of Subjects

We will attempt to retain program participants once randomized at a retention rate of 80% for study completion through Day 42 and close-out visit. It is our desire to analyze results on all participants who were included into the program (e.g. completed SV1 visit). In accordance with the declaration of Helsinki/Tokyo/Venice/Hong Kong, participants have the right to withdraw from the program at any time for any reason. The investigator also has the right to withdraw participants from the program treatments in the event of inter-current illness, adverse experience, treatment failure, protocol violation, or other reasons. Should a participant decide to withdraw from treatment, all efforts will be made to complete and report follow-up observations as thoroughly as possible.

Risks to Subjects

This study does not involve major risk to screeners and trial participants. Efforts to minimize the potential risks of the assessment methods and outcome variables include frequent monitoring by the investigators to assure that no participant suffers any adverse effects from participating in the research. The study procedures include:

- $^{2}H_2O$ (i.e., heavy water). The extra neutron in the heavy water is not radioactive and has no risk. Children and pregnant women have been given this "special" water.
- Body weight. There is no risk to participants who record their body weight.
- Blood Pressure Testing. Participants may experience temporary discomfort during blood pressure recordings due to the pressure of the cuff on their arm.
- Venipuncture (blood draw). There is the possibility of pain and bruising at the vein on the participant's arm where the needle is inserted. Aseptic (sterile) technique and trained personnel minimize these risks.
- Venous catheterization. There is a possibility of pain, bruising, or infection at the site of the needle insertion for the IV line. Trained personnel minimize this risk.
- Dual Energy X-ray Absorptiometry (DXA). The amount of radiation used for this procedure is very small. The radiation dose for this scan is equivalent to the radiation an individual is naturally exposed to in the environment in less than one day. The completion of 6 scans over the course of the trial is 500 times below the limit for radiation exposure per year. Per Pennington SOP, the quantity of scans completed in the trial is within the 10 DXA scans per year limit.

- Functional magnetic resonance imaging (fMRI). There are no known biological risks associated with magnetic resonance scanning. It has been used routinely for over 20 years. It produces side effects in very few situations. Those situations include:

    - **Metal**: Because the magnetic resonance machine uses a magnetic field, it can move any metallic objects that are inside the body. *This disruption of metal inside the body is extremely dangerous and may even be life threatening.* If the participant thinks he/she may have a cardiac stent, metallic implant, metallic piercings, shrapnel, or any other metallic material in the body, it is of utmost importance that the participant alert the study coordinator or MR technician. If the participant has metallic materials in the body that cannot be removed, we will exclude the participant from this study.
    - **Electronics**: Magnetic resonance imaging involves the use of radio frequency energy that can disrupt the functioning of electronic devices. If the participant possesses a pacemaker or any other electronic medical device inside the body, the participant will be excluded.
    - **Tattoos and cosmetics**: Some tattoos and cosmetics contain metallic materials that can heat up during scanning, especially if they are located on the part of the body being scanned. If the metallic material heats up enough, the participant may feel an uncomfortable burning sensation, and a skin burn may develop. In some cases, the amount of metallic material in the area being scanned is so excessive that the scan cannot proceed without risk of a burn developing. In other cases, a cold compress placed over the metallic material can be used to prevent burning.
    - **Confinement**: During the MR scan, the participant will be lying down on a table inside of a metal tube. The metal tube is a confined place. This might produce a feeling of claustrophobia, which can be distressing. A participant who has experienced claustrophobia in the past might become too distressed to complete the scan. In this case, the scan will be halted.
    - **Noise:** The MRI machine creates a loud, rhythmic noise that sounds like grinding or churning. This can be distressing to those who are sensitive to loud noises. The participant will be provided with earplugs to reduce the noise. But, if the participant finds the machine noises distressing, the MR technician can halt the scan.
    - **Peripheral nerve stimulation:** During the MR scan, the magnetic field around the body goes through rapid changes. These changes are all within safety limits set by the Food and Drug Administration. But, some people experience twitching in the nerves of their arms or legs as a result of these magnetic field changes. This twitching is generally not painful, and it stops at the end of the MR scan. But the feeling of inadvertent muscle twitching may make individuals feel disoriented or uncomfortable. Any participant who experiences this and wishes to stop the scan as a result will be allowed to do so.
    - **Electrical stimulus:** The BioPac device providing painful electrical stimulus has been safely applied in human subjects under a variety of conditions. Although the experimental design requires that the stimulus be painful, the electrical stimulation has no physical effects that last

beyond the duration of the task. There is a risk that the application of a painful stimulus may be psychologically distressing to the participant. In this event, the task will be terminated.
  - **Mental tasks performed during fMRI:** There are no anticipated risks from performing these tasks.

- Self-report Questionnaires. There are no anticipated risks from completing self-report questionnaires. If signs of minor stress or fatigue are apparent, participants will be given time to take a break from completing the questionnaires. It is estimated that the questionnaires will take from 90 to 120 minutes to complete. The questions contained in some of the questionnaires may make people feel uncomfortable since they ask about topics such as how they feel about their body size. Responses to the questions will be coded to protect confidentiality, and participants may choose to not answer questions.
- Accelerometry. There is no risk associated with measuring activity with accelerometers. Accelerometers fit comfortably on the participant's arm and at the waist and can easily be removed should they become uncomfortable.
- Archive of Biological (Blood) Sample. The primary risk to participants regarding blood to be banked for future research is the risk of loss of confidentiality and/or privacy. Most banks need to maintain a link between the identities of donors and coded specimens to be able to collect valuable clinical follow-up information about the donor. To insure participants' privacy and confidentiality, their samples will be labeled with a unique series of letters and numbers. Pennington Biomedical will store these samples with unique identifiers and a minimum number of personal identifiers to meet laboratory standards. Storage and disposal of tissue will be conducted in a manner conforming to the appropriate care and handling of biological specimens as outlined through the Institutional Biohazard Committee Guidelines.
- Muscle Biopsy. Mild to severe pain, soreness, bruising, and a small scar are common risks. A hematoma (collection of blood in the tissue)) may occur. There is a slight risk that a superficial nerve may be cut; the nerve may heal, or it may result in a permanent loss of sensation in the skin at the biopsy site. Although infrequent, there is risk of infection at the biopsy site, which may need treatment with antibiotics.
- Metabolic Chamber. A participant may experience some level of claustrophobia or discomfort from staying in the chamber and being continuously monitored by a camera. However, participants will not be locked in and will be able to open the door in case of an emergency. The camera has been installed for participants' safety and no one is allowed access to the monitor except chamber personnel.
- 24 Hour Urine Collection. There are no known risks of collecting urine into a container.
- Fecal Collection. There are no known risks of collecting a fecal sample.
- Consumption of D_3-creatine (D_3Cr) (60 mg capsule). This is a stable isotope, and thus is not radioactive. Consuming this isotope at levels described in this study is considered safe.
- Testosterone. Potential side effects of testosterone treatment include acne, oiliness of skin, increased growth of body hair, breast tenderness, a reversible increase in hemoglobin, sleep apnea, leg edema and weight gain.
- EKG. There are minimal risks associated with this test. There is a small possibility there may be some redness or irritation while cleaning the skin prior to applying the

electrodes or if a participant happens to be allergic to the adhesive on the electrodes.

- Exercise testing. There is minimal risk of injury or a cardiovascular event during exercise testing. We believe the risk of an event during exercise testing is minimized with a pretest review of the medical history, physical examination by a physician or mid-level health care professional, use of a highly trained staff, and well-defined emergency procedures. Participants may experience temporary discomfort during blood pressure recordings due to the pressure of the blood pressure cuff on the arm. All tests are conducted in the presence of an exercise physiologist with extensive experience in conducting maximal exercise tests as well as a Research Associate specializing in cardiology. All laboratory staff are trained in basic CPR and/or ACLS (advanced cardiac life support). In the event of a life threatening emergency, the subject would be treated with ACLS by a staff M.D. and research nurses and subsequently be transported to the nearest acute care medical-surgical facility via Emergency Medical Services which is a parish wide paramedic response unit. The closest facility is approximately 0.25 miles away.
- Exercise interventions. The proposed exercise interventions are unlikely to cause major problems. We have conducted numerous exercise training studies and have never had a serious adverse event. There is the possibility of adverse events ranging from minor musculoskeletal problems to, in very rare cases, cardiovascular events. Exercise bouts will include a variety of modes (walking, cycling, elliptical, running, and pack walking) and intensities (low to high) at the discretion of the trainer to ensure compliance to the intervention as well as minimizing injury. Occasionally study participants experience minor orthopedic problems, but most are self-correcting with rest and standard first aid. These orthopedic injuries will be minimized by gradually progressing participants to their prescribed dose at the start of the study and alternating exercise sessions between cycle ergometers and treadmills as necessary. Exercise supervisors are trained in first aid and basic CPR. Each staff member is trained in either advanced or basic life support, and an automated external defibrillator and fully-stocked crash cart are kept on site. Although some study participants will be at moderately elevated risk for CVD, they will receive a thorough health screen including a physical examination by a study physician or mid-level health care professional and a maximal exercise test. According to the available data on adverse events resulting from the types of exercise proposed here, risk should be low in this study. Fatal events during exercise are extremely rare.
- Energy Deficit. There is a small risk of hypoglycemia when starting a diet lower in calories than participants are accustomed to and increasing caloric expenditure via exercise. Regular blood work and monitoring (including vitals) will be completed throughout the phases for safety checks. Prior to inclusion in the trial, all participants are screened to exclude those with medical or mental disorders that are unsuitable for the trial. Additionally, a barriers interview will be completed during screening to further explain study demands, assess barriers to participation, and screen for psychological and behavioral contraindications to the trial. The clinical site physician will review the medical procedures and psychological health of each participant and attest to suitability for inclusion to into the trial. Once enrolled in the trial, study staff with psychological and behavioral expertise will make daily rounds on the unit to assess mental stability and health of the participants. Any participant

displaying undue distress or other negative psychological symptoms as a result of the trial procedures, the PI and MI will assess if continuation in the trial is warranted. An on-call physician is also available after hours and on weekends.

Potential Benefits to Subjects

No direct benefits to the participants are expected.

Sharing of Results with Subjects

Participants will be provided a summary results sheet at the completion of their participation in the study. The summary results will include body composition andphysical fitness tests, and available lab work results.

The MR scan data collected for the study is not designed to be used by a doctor to evaluate physical health the way a scan done in a hospital or clinic is. That means that any abnormalities in the body that are relevant to personal health will not necessarily be noticed by study personnel. However, MR scanning may result in discovery of an unexpected incidental finding as described above. Therefore, there is a small chance that this study will provide benefit by revealing problems with personal health that would not have been discovered without MR scanning.

Setting

Clinical Facility

A detailed description of the clinical research facilities at Pennington Biomedical can be found in **Appendix M**.

Exercise Facility

The PBRC site has a 2,300 square foot Exercise Training Facility, which is under the management of the Preventive Medicine Department. Dr. Church is the Medical Director of this core. The facility offers state-of the-art equipment, professional intervention technicians, and optional training data capturing capabilities. The cardiovascular fitness training room contains treadmills, stationary bikes, and ellipticals. Multiple televisions hang from the ceilings in each room and a variety of magazines are available for use by the participants. Locker rooms are equipped with lockers, showers, and towels. Calibrated scales are available to measure body weight and work stations with computers are available that provide a private area to meet with participants. A dedicated parking lot with handicap parking is located immediately outside of the facility. The fitness center is supported by a trained staff composed of full-time technicians, exercise interventionists, post-doctoral researchers and internship students working on exercise-related degrees. Each is trained in either advanced or basic life support, and an automated external defibrillator.

Compensation

We will provide $6,000 per individual as an incentive for participation in the study. We think this amount is appropriate given the amount of time and procedure burden that the participants will spend both during the free living phase and live-in diet phase.

Participants will receive $500 after the completion of screening and free-living diet acclimation (randomization at day 14). They will also receive $3500 for completion of the live-in diet and activity phase (day 15 through day 42). If the participant does not complete the entire live-in diet phase, the compensation will be prorated based on the number of days completed. Lastly, participants will receive $2000 for completion of the free-living phase (close out visit). Total compensation will be up to $6000.

Confidentiality

All participants are assured of their confidentiality both verbally and in the informed consent form. The clinical facilities are strictly limited to the staff of the research institution and to research participants. This is accomplished by a variety of stringent security measures. All medical records are stored in locked areas. Access to these areas is limited to the clinical support staff, director of the clinical facilities, and the PIs. Participants' medical records are filed according to ID numbers. All forms on the chart display the ID number. Electronic data storage is similarly restricted with only the PIs and authorized persons having access to databases containing confidential clinical records, i.e. those containing name OR other identifying information.

Data, including body weight, body composition, exercise testing, etc. will be collected from participants. Data are confidentially collected from study participants and are only used for research purposes. All records are kept in locked file cabinets, and participant data can be identified only by number. Data are used only in aggregate, and no identifying characteristics of individuals are published or presented.

The MRI screening form contains potentially sensitive information about the health condition of the participant. These forms are considered study records and are kept in Medical Records or in a locked cabinet in the MRI Suite. Each participant is assigned a numerical identifier that is assigned to all MRI files collected as part of the study. All PHI is removed from these files when the numerical identifier is assigned to them. The mapping from subject name to numerical identifier is maintained by the Research Computing Group.

Data Sharing

The participant is asked to allow their study data to be stored and used for research at a later time. Participants that refuse to have study data kept for future research will be excluded from the study. The study data will be stored indefinitely. The data may be given to other investigators for future research as well. The future research may take place at Pennington Biomedical and may involve Pennington Biomedical Researchers in this study. The future research may not take place at Pennington Biomedical Research Center and may not be reviewed by Pennington Biomedical Research Center's Institutional Review Board. For privacy and confidentiality, study data will be labeled with a unique series of letters and numbers. Pennington Biomedical will store study data with this unique identifier. The research done with study data may help to develop new products in the future, or may be used to establish a diagnostic test that could be patented or licensed. The participant will not receive any financial compensation for any patents, inventions or licenses developed from this research.

Incidental Findings

We will follow the recommendations of a Presidential panel in handling incidental findings on MRI [132]. First, we indicate to the participant whether or not there is a well-established set of incidental findings for the scan they are undertaking, and if so what those incidental findings are. For scans without a well established set of incidental findings, we will provide a list of incidental findings that we feel may be possible. We will then describe the difference between clinically actionable incidental findings and non-clinically-actionable incidental findings. We will then ask the participant to decide whether they want to be informed of non-clinically-actionable incidental findings. Participants will be informed that informing them of clinically-actionable incidental findings is required for participation in the study. In the event that study personnel identify MR abnormalities, they will consult with a radiologist who will determine the clinical relevance of the abnormalities. Participant identity will not be shared with the radiologist in this event. If the radiologist determines that an MR abnormality is relevant to personal health, the radiologist will then determine whether the finding is clinically actionable. In the event that the finding is clinically actionable, or if the participant consented to be informed of non-clinically actionable incidental findings, study personnel will provide the information to the study Medical Investigator so that he or she can discuss the relevance of the finding with the participant. In the event of an incidental finding that is to be released to the participant, the imaging findings flow from the study staff, to a radiologist, to the Medical Investigator who explains the findings with the participant.

Compensation for Research-Related Injury

In the unlikely event a participant becomes injured as a result of participation in this study, medical care is available. It is the policy of this institution to provide neither financial compensation nor free medical treatment for research-related injury. By participating in this study, participants are not waiving any rights they have against PBRC/USARIEM for injury resulting from negligence of PBRC/USARIEM or its investigators.

Roles and Responsibilities

Principal Investigator (PBRC)

The Principal Investigator (PI) will assist in all aspects of the study including scheduling, briefing potential volunteers, being responsible for record keeping, quality assurance issues, maintenance of confidentiality, and notification to the DSMB in the event of an adverse incident. Procedures will be performed only by privileged personnel or by personnel who are under direct supervision of the privileged personnel.

Jennifer C. Rood, Ph.D.: Dr. Rood will be the PI of record and assume responsibility for the safe and scientifically sound conduct of the study. She will oversee all aspects of the study, assist with data collection, ensure safety and ethical treatment of participants, maintain required documentation for the study, obtain required approvals, have primary responsibility for data analysis and assist with data interpretation and publication.

Co-Investigators (USARIEM)

USARIEM co-investigators will be non-engaged, as they will not obtain: information about participants of the research through intervention or interaction with them, identifiable private information about the participants of the research, or the informed consent of human subjects involved in the research. All data files and biological samples (muscle, blood, urine, fecal samples) sent from PBRC to USARIEM will be de-identified. USARIEM_co-investigators will assist with concept development, formulation of protocol objectives, hypotheses, experimental approach and design. Co-investigators will maintain active communication with the PI and study staff.

Stefan M. Pasiakos, Ph.D. and Claire E. Berryman, Ph.D.: Drs. Pasiakos and Berryman will assist with concept development, formulation of protocol objectives, hypotheses, experimental approach and design. They will have primary responsibility for data interpretation and preparation of manuscripts and technical reports for publications. Drs. Pasiakos and Berryman will share primary responsibility for all communication with PBRC and routinely visit PBRC to ensure study progress and proper execution of the project.

APPENDICES

Appendix A: Train schedule
Appendix B: AR 600-9 (Army Body Comp Program)
Appendix C: Semi-structured barriers interview
Appendix D: 3-d food diary and instructions
Appendix E: Blood measures
Appendix F: Example PT circuit
Appendix G: 6-d cycle menu example
Appendix H: Daily food diary
Appendix I: Saliva collection instructions and log
Appendix J: Biodex performance data form
Appendix K: Questionnaires, evaluations, cognitive performance tests, and personality assessments
Appendix L: Fecal collection instructions
Appendix M: Detailed Description of the Clinical Research Facilities at Pennington Biomedical Research Center

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	Screening			Phase I: Diet Acclimation (free-living)																								Phase
Procedure	Phone/ Web Screen	Scr 1	Scr 2	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25
Phone/Web Screen	x																												
Informed Consent		x																											
Height		x																											
Weight		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Head Circumference			x																										
Neck/Waist Circumference		x																											
Medication Use		x	x																										
Barriers Interview			x																										
BP, Pulse		x	x																x	x	x	x	x	x	x	x	x	x	x
Med. Hx		x																											
Physical Exam			x																										
ECG			x																										
Chem 15				x														x											
Chem 26			x																										
CBC			x	x														x											
Urinalysis			x																										
Urine Drug Screen			x																										
Blood Archive			x	x							x							x											
Urine Collection			x												x		x	x											
Blood Draw			x	x							x							x											
Saliva Collection							x				x				x				x				x				x		
Testosterone			x	x														x											
Free testosterone			x	x														x											
LH				x														x											
FSH				x														x											
SHBG				x														x											
IGF-1				x														x											
Estradiol				x														x											
Insulin				x							x							x											
Cortisol				x														x											
Oxytocin				x														x											
DHEA-s				x														x											
Prolactin				x														x											
Vasopressin				x														x											
Melatonin				x														x											
PSA			x															x											
Lp(a)				x														x											
Amino Acids				x														x											
Hepcidin				x														x											
IL-6				x														x											
sTFR				x														x											
Ferritin				x														x											
Lipopolysaccharide Binding Protein				x														x											
PARQ		x																											
CVD risk stratification assessment		x																											

Adverse Events			x	x						x							x							x				
Cognitive Training				x		x		x			x			x														
Cognitive Testing									x								x		x					x		x		
MMPI									x																			
Buss-Perry, POMS, SLIM, BART, Ultimatum, RTMITE, SVVT, PVT, Matching, Grammatical Reasoning, N-back				x													x		x					x		x		
DXA				x										x														
VO2 Max				x																								
fMRI+									x				x			x												
Task 1 & 3									x																			
Task 4 & 5													x															
Task 2 & 6																x												
Biodex, Familarization test				x																								
Biodex																	x											
Bike, Familarization Test*					x*																							
Exercise (60 min Bike)																			x									
24 hr urine nitrogen														x														
24 hr urine creatinine														x														
Metabolic Chamber														x														
Musc Biopsy (rest)																			x									
Muscle Biopsy (exercising)																			x									
Randomization																			x									
^2H_2O				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
D_3-Cr														x														
3 day food record			x																									
Activity Log			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x											
Eating Inventories (TFEQ, FCI, FCI Trait)			x														x											
Wrist Worn ReadiBand				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Actigraph Accelerometry			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x											
Fecal Collection														x														x
Gut Microbiome														x														x
Injection																		x						x				
Meals/Snacks Outpatient				x	x	x	x	x	x	x	x	x	x	x	x	x												
Meals/Snacks Inpatient														x			x	x	x	x	x	x	x	x	x	x	x	x
Supervised Exercise Intervention																		x	x	x	x	x	x	x	x	x	x	x
Kitchen Screening		X																										
Food Intake Test										x																		
VAS										6																		
Leptin										x																		
Glucose										5																		
Active Ghrelin										5																		
Inactive Ghrelin										5																		
PYY										5																		
GLP-1										5																		
Urine Mannitol														x														
Urine Sucralose														x														
Sugar Ingestion Test														x														

*BF Bike Familiarization Ride will be completed anytime between Day 1 and Day 8																							

e 2: 55% Energy Deficit (live-in diet & activity control)

Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33	Day 34	Day 35	Day 36	Day 37	Day 38	Day 39	Day 40	Day 41	Day 42	Day 43	Day 44	Day 45	Day 46	Day 47	Day 48	Day 49	Day 50	Day 51	Day 52	Day 53	Day 54	Day 55	Day 56	Day 57
x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x															
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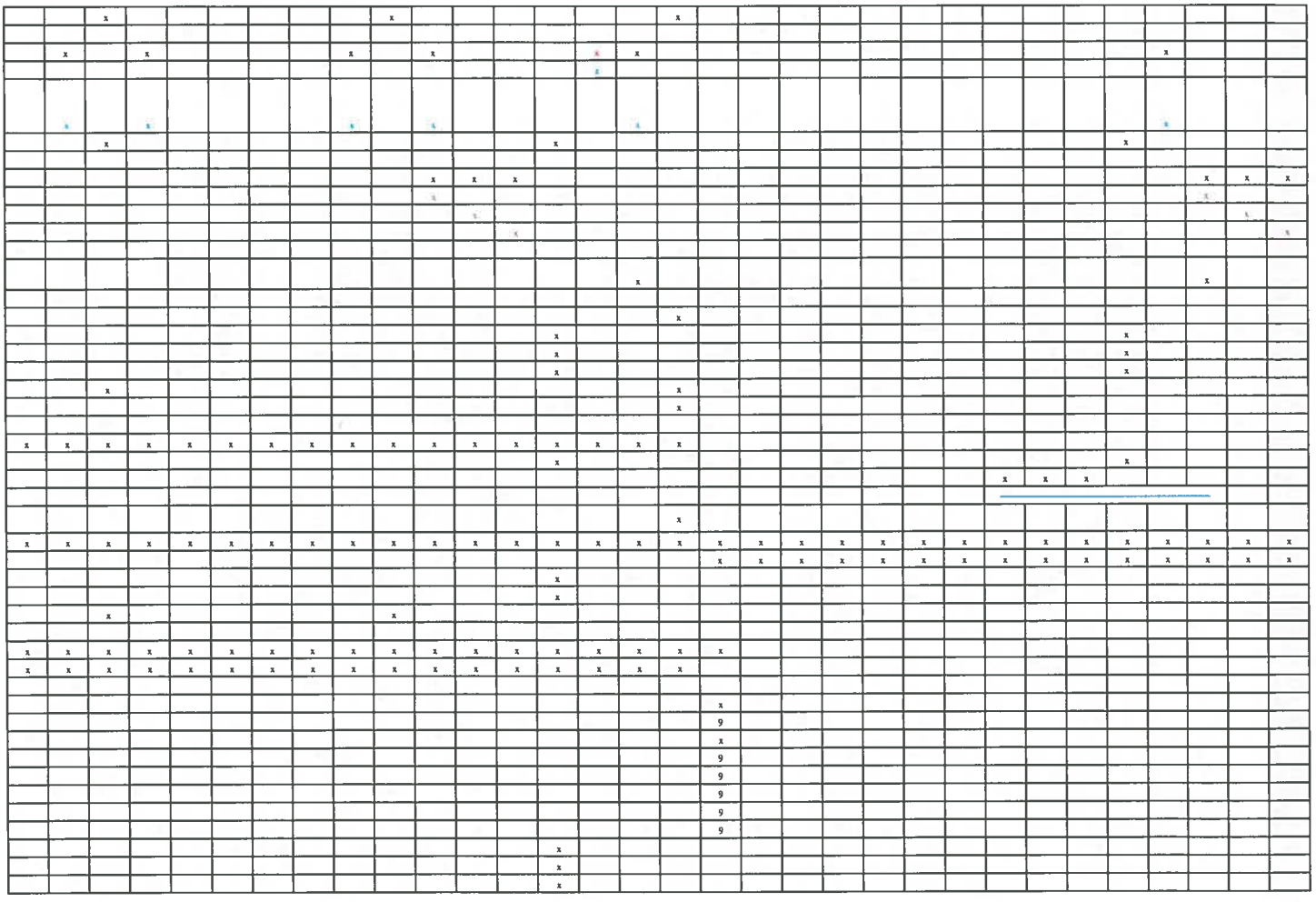

Pennington Biomedical IRB FWA 00006218
Approved 12/09/2016

Phase 3: Ab Libitum Feeding (free-living)																											End of Study			
Day 58	Day 59	Day 60	Day 61	Day 62	Day 63	Day 64	Day 65	Day 66	Day 67	Day 68	Day 69	Day 70	Day 71	Day 72	Day 73	Day 74	Day 75	Day 76	Day 77	Day 78	Day 79	Day 80	Day 81	Day 82	Day 83	Day 84	End of Study	EOS +1 Days	EOS +2 Days	EOS +3 Days
x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
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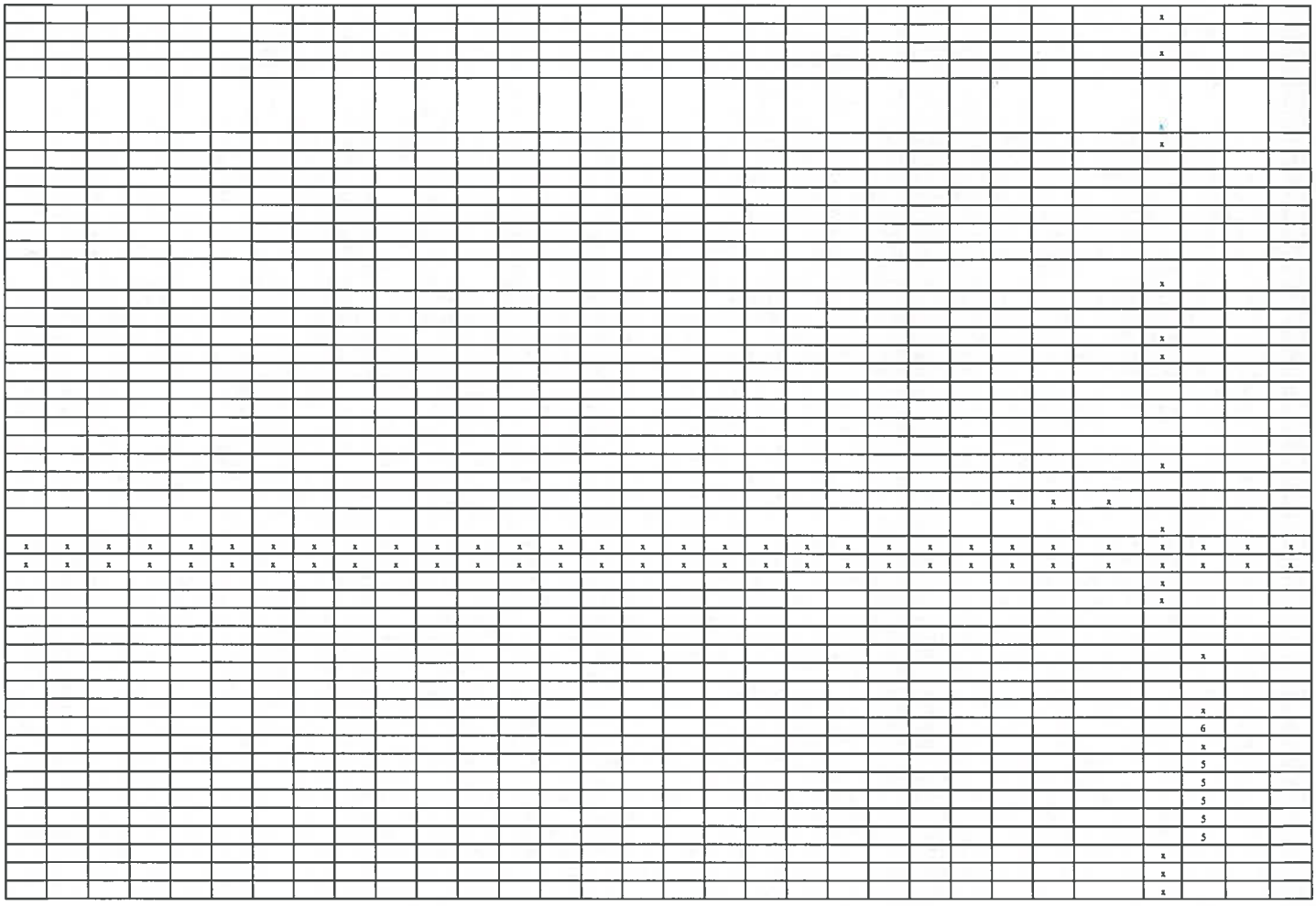

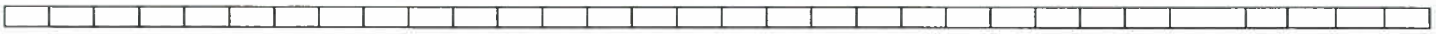

Army Regulation 600–9

Personnel-General

The Army Body Composition Program

Headquarters
Department of the Army
Washington, DC
28 June 2013

UNCLASSIFIED

SUMMARY of CHANGE

AR 600-9
The Army Body Composition Program

This major revision, dated 28 June 2013-

- Changes the name of the regulation from the Army Weight Control Program to the Army Body Composition Program (title page and throughout).
- Adds responsibility for Deputy Chief of Staff, G-3/5/7 (para 2-4).
- Replaces U.S. Army Reserve Components Personnel Center with U.S. Army Human Resources Command (para 2-13).
- Deletes requirement to establish an interim process to collect and maintain data for submission in an annual report (para 2-16).
- Deletes specific procedures required prior to attendance at institutional training; clarifies suspension of favorable personnel action (Flag) process to align with current policy (chap 3).
- Deletes specific procedures related to bars to reenlistment and administrative separations (para 3-2).
- Exempts certain categories of Soldiers from meeting the requirements of this regulation, with the exception of the requirement to maintain a Soldierly appearance (para 3-3).
- Replaces medical holding units with Warrior Transition Unit or Community Based Warrior Transition Unit (para 3-3*a*).
- Adds time frames for specific actions, Army Body Composition Program enrollment, counseling, and evaluations for Soldiers (paras 3-6, 3-7, and table 3-1).
- Updates definition of Army Body Composition Program progress to include 1 percent body fat loss per month (para 3-9*b*).
- Clarifies procedures for Soldiers with a temporary medical condition (para 3-11).
- Defines the Army Body Composition Program failure as 3 nonconsecutive months of less than satisfactory progress (para 3-12).
- Clarifies procedures to request an exception to policy (para 3-17).
- Requires weight scale calibration annually (para B-2*b*).
- Updates weight loss information (app C).
- Updates figures and terminology (throughout).

Headquarters
Department of the Army
Washington, DC
28 June 2013

*Army Regulation 600–9

Effective 28 July 2013

Personnel-General

The Army Body Composition Program

By Order of the Secretary of the Army:

RAYMOND T. ODIERNO
General, United States Army
Chief of Staff

Official:

GERALD B. O'KEEFE
Acting Administrative Assistant
to the Secretary of the Army

History. This publication is a major revision. The portions affected by this major revision are listed in the summary of change.

Summary. This regulation implements guidance in Department of Defense Instruction 1308.3, which implements policy and prescribes procedures governing physical fitness and weight/body fat standards in the Services.

Applicability. This regulation applies to the Active Army, the Army National Guard/Army National Guard of the United States, and the U.S. Army Reserve, unless otherwise stated.

Proponent and exception authority. The proponent of this regulation is the Deputy Chief of Staff, G–1. The proponent has the authority to approve exceptions or waivers to this regulation that are consistent with controlling law and regulations. The proponent may delegate this approval authority, in writing, to a division chief within the proponent agency or its direct reporting unit or field operating agency in the grade of colonel or the civilian equivalent. Activities may request a waiver to this regulation by providing justification that includes a full analysis of the expected benefits and must include formal review by the activity's senior legal officer. All waiver requests will be endorsed by the commander or senior leader of the requesting activity and forwarded through their higher headquarters to the policy proponent. Refer to paragraph 3–17 and AR 25–30 for specific guidance.

Army internal control process. This regulation contains internal control provisions in accordance with AR 11–2 and identifies key internal controls that must be evaluated (see appendix D).

Supplementation. Supplementation of this regulation and establishment of command and local forms are prohibited without prior approval from the Deputy Chief of Staff, G–1 (DAPE–HR), 300 Army Pentagon, Washington, DC 20310–0300.

Suggested improvements. Users are invited to send comments and suggested improvements on DA Form 2028 (Recommended Changes to Publications and Blank Forms) directly to Deputy Chief of Staff, G–1 (DAPE–HR), 300 Army Pentagon, Washington, DC 20310–0300.

Distribution. This publication is available in electronic media only and is intended for command levels A, B, C, D, and E for the Active Army, the Army National Guard/Army National Guard of the United States, and the U.S. Army Reserve.

Contents (Listed by paragraph and page number)

*This regulation supersedes AR 600–9, dated 27 November 2006.

Contents—Continued

Appendixes

Table List

Contents—Continued

Figure List

Glossary

Chapter 1
Introduction

1–1. Purpose
This regulation establishes policies and procedures for the implementation of the Army Body Composition Program (ABCP).

1–2. References
Required and related publications and prescribed and referenced forms are listed in appendix A.

1–3. Explanation of abbreviations and terms
Abbreviations and special terms used in this regulation are explained in the glossary.

1–4. Responsibilities
Responsibilities are listed in chapter 2.

1–5. Objectives
a. The primary objective of the ABCP is to ensure all Soldiers achieve and maintain optimal well-being and performance under all conditions.

b. Secondary objectives of the ABCP are to—

(1) Assist in establishing and maintaining—

(a) Operational readiness.

(b) Physical fitness.

(c) Health.

(d) A professional military appearance in accordance with Army Regulation (AR) 670–1.

(2) Establish body fat standards.

(3) Provide procedures by which personnel are counseled to assist in meeting the standards prescribed in this regulation.

Chapter 2
Responsibilities

2–1. General
Soldiers must maintain a high level of physical readiness in order to meet mission requirements. Body composition is one indicator of physical readiness that is associated with an individual's fitness, endurance, and overall health. Individuals with desirable body fat percentages generally exhibit increased muscular strength and endurance, are less likely to sustain injury from weight bearing activity, and are more likely to perform at an optimal level. Soldiers will meet Army body composition standards, as prescribed in this regulation, for the individual and collective benefit to themselves, their unit, and the entire Army.

2–2. Deputy Chief of Staff, G–1
The DCS, G–1 is responsible for the ABCP.

2–3. The Surgeon General
The Surgeon General will—

a. Establish medical examination and medical counseling policies in support of the ABCP.

b. Evaluate the medical aspects of the program.

c. Establish and review procedures for determination of body fat content.

d. Provide guidance on improving the nutritional status of Soldiers.

e. Provide recommendations and/or medical opinions on medical exception to policy requests to the Office of the DCS, G–1.

2–4. Deputy Chief of Staff, G–3/5/7
The DCS, G–3/5/7 will establish training guidance in support of the ABCP.

2–5. Deputy Chief of Staff, G–4
The DCS, G–4 will—

a. Establish food service guidance in support of the ABCP.

b. Publish guidance and information pertaining to the performance nutrition contribution of items served on master menus.

2–6. Chief, National Guard Bureau

The Chief, National Guard Bureau will—

a. Implement and monitor the ABCP in the Army National Guard (ARNG).

b. Take appropriate action under guidance prescribed in this regulation.

2–7. Chief, Army Reserve

The Chief, Army Reserve will—

a. Monitor the ABCP in the U.S. Army Reserve (USAR).

b. Take appropriate action under guidance prescribed in this regulation.

2–8. Commanding General, U.S. Forces Command

The CG, U.S. Army Forces Command will implement and monitor the ABCP in Active Component (AC) units and USAR to include troop program units, reinforcement training units, and continental United States individual mobilization augmentees.

2–9. Commanders of Army commands, Army service component commands, and direct reporting units

The commanders of ACOMs, ASCCs, and DRUs will ensure that Soldiers within their commands are evaluated under the body fat standards prescribed in this regulation.

2–10. Commanding General, U.S. Army Training and Doctrine Command

The CG, U.S. Army Training and Doctrine Command is responsible for ensuring Soldiers are trained on basic performance nutrition at the time of their initial entry.

2–11. School commandants

U.S. Army Training and Doctrine Command school commandants, and commandants and/or commanders of USAR Forces schools, the Army Reserve Readiness Training Center, and/or ARNG-conducted schools (regional noncommissioned officer (NCO) academies, State military academies, or ARNG professional education center courses) will take the actions in accordance with AR 350–1 upon determining that a student arrived for a professional military school who exceeds the body fat standard.

2–12. Commanding General, U.S. Army Medical Command

The CG, U.S. Army Medical Command will—

a. Establish and provide weight reduction and counseling programs in Army medical treatment facilities (MTFs) in support of the ABCP.

b. Provide appropriate literature and training aids for use by Soldiers, supervisors, and commanders in selection of a proper diet.

c. Ensure commanders of overseas major medical commands institute weight reduction and counseling programs in Army medical facilities in support of the ABCP.

2–13. Commanding General, U.S. Army Human Resources Command

The CG, U.S. Army Human Resources Command will—

a. Monitor the ABCP in the Individual Ready Reserve (IRR).

b. Take appropriate action under guidance prescribed in this regulation.

c. Ensure that members applying for tours of active duty, active duty for training (ADT), active duty support, and Active Guard Reserve (AGR) meet the body fat standards prescribed in this regulation. Soldiers who do not meet these standards will not be permitted to enter on active duty, ADT, active duty support, or in AGR status.

2–14. Individuals

Each Soldier (commissioned officer, warrant officer, and enlisted) is responsible for meeting the standards prescribed in this regulation.

2–15. Order issuing officials

Order issuing officials will ensure all temporary duty and permanent change of station orders include the following in the text: "You are responsible for reporting to your next duty station and/or school in satisfactory physical condition, able to pass the Army Physical Fitness Test (APFT), and meet body fat standards in accordance with AR 600–9."

2–16. Commanders and supervisors

Commanders and supervisors (Active Army and Reserve Component (RC)) will—

a. Implement the ABCP, to include evaluation of the military appearance of all Soldiers under their jurisdiction and measurement of body fat as prescribed in this regulation.

b. Ensure the continued evaluation of all Soldiers under their command or supervision against the body fat standards prescribed in this regulation.

c. Review monthly Suspension of Favorable Personnel Actions Management Report (AAA–095) for all Soldiers who are flagged or have been flagged within the past 36 months for failing to meet body fat standards.

d. Forward a complete ABCP file (see para 3–8) to the gaining unit on each Soldier who conducts a permanent change of station and is flagged for noncompliance with body fat standards.

2–17. Health care personnel

Health care personnel will—

a. Assist commanders and supervisors in ensuring that individuals who exceed body fat standards receive nutrition and weight reduction counseling from a registered dietitian, if available. If a registered dietitian is not available, nutrition and weight reduction counseling may be provided by a health care provider, to include nurse practitioner, physician assistant, or medical doctor.

b. Identify those individuals who have a pathological condition requiring medical treatment.

c. Evaluate Soldiers who exceed body fat standards in accordance with this regulation.

d. Advise Soldiers that while various medical conditions, environmental conditions, functional limitations (temporary or permanent physical profiles), and/or medications may contribute to weight gain, they are still required to meet the body fat standard established in this regulation. The DCS, G–1 is the exception to policy approval authority (see para 3–17) for special considerations.

e. Refer Soldiers to appropriate specialist for nutrition and exercise counseling, if indicated.

f. At the request of a commander, provide education and information to Soldiers on healthy eating behaviors.

2–18. Designated unit fitness training noncommissioned officer or master fitness trainer

A designated unit fitness training NCO or master fitness trainer will—

a. Prescribe proper exercise and fitness techniques, according to Field Manual (FM) 7–22, to assist Soldiers in meeting and maintaining body fat standards.

b. Assist commanders in developing programs that establish a physical fitness program in accordance with FM 7–22.

c. Train other command designated NCOs in proper height, weight, and body circumference methodology to assess body fat composition.

Chapter 3
Army Body Composition Program

3–1. Overview

Soldiers are subject to many demands and challenges that may impact individual readiness. The ABCP provides commanders a systematic approach to enforce military standards across the unit, while supporting Soldiers with the resources they need to return to an optimum level of individual readiness.

3–2. Standard

a. Soldiers are required to meet the prescribed body fat standard, as indicated in appendix B. Soldiers will be screened every 6 months, at a minimum, to ensure compliance with this regulation.

b. The only authorized method of estimating body fat is the circumference-based tape method outlined in appendix B.

c. Commanders are authorized to use the weight for height table (see app B) as a screening tool in order to expedite the semi-annual testing process. If Soldiers do not exceed the authorized screening table weight for their age and measured height, no body fat assessment is required.

d. Commanders have the authority to direct a body fat assessment on any Soldier that they determine does not present a Soldierly appearance, regardless of whether or not the Soldier exceeds the screening table weight for his or her measured height.

e. Soldiers identified as exceeding the body fat standard will be flagged in accordance with AR 600–8–2 and enrolled in the ABCP. They must meet the body fat standard in this regulation in order to be released from the program.

3–3. Exemptions

a. Soldiers assigned or attached to a Warrior Transition Unit or Community Based Warrior Transition Unit must meet the body fat standard. Soldiers with special considerations may request a temporary exception to policy. See paragraph 3–17.

b. The following Soldiers are exempt from the requirements of this regulation; however, they must maintain a Soldierly appearance:

(1) *Soldiers with major limb loss.* Major limb loss is defined as an amputation above the ankle or above the wrist, which includes full hand and/or full foot loss. It does not include partial hand, foot, fingers, or toes.

(2) *Soldiers on established continued on active duty and/or continued on active Reserve status.* See AR 635–40.

(3) *Pregnant and postpartum Soldiers.* See paragraph 3–15.

(4) *Soldiers who have undergone prolonged hospitalization for 30 continuous days or greater.* See paragraph 3–16.

(5) *New recruits.* These recruits, regardless of component, will have 180 days from entry to active service to meet the retention body fat standards established in this regulation. Failure to achieve retention body fat standards at 180 days will result in Soldiers being flagged in accordance with AR 600–8–2 and enrolled in the ABCP.

c. Soldiers that do not meet the criteria of paragraph *b*, above have the option to request a temporary exception to policy. See paragraph 3–17.

3–4. Weigh-in and body fat assessment

a. Weigh-ins and body fat assessments will be conducted in accordance with appendix B. All Soldiers will be weighed every 6 months, at a minimum.

b. In order to ensure the ABCP does not interfere with Soldier performance on the APFT, commanders and supervisors are encouraged to allow a minimum of 7 days between APFT and weigh-in, if feasible. Some Soldiers that are close to exceeding the screening weight may attempt to lose weight quickly in the days leading up to a weigh-in. This practice may result in the Soldier being unable to perform his or her best on the APFT, if the two events are scheduled close together.

c. Routine weigh-ins will be accomplished at the unit level. Percent body fat assessments will be accomplished by company or similar level commanders (or their designee) in accordance with standard methods prescribed in appendix B. Soldiers will be measured by trained individuals of the same gender. If a trained individual of the same gender is not available to conduct the measurements, a female Soldier will be present when a male measures a female, and a male Soldier will be present when a female measures a male. IRR members on annual training, ADT, and special ADT will have a weigh-in and body fat assessment (if required) by the unit to which they are attached.

d. Units maintain height, weight, and body fat assessment data according to unit policy. The height, weight, and body fat percent may be entered on the Department of the Army (DA) Form 705 (Army Physical Fitness Test Scorecard) but they are no longer required entries. Units may track height and weight on a centralized roster, the DA Form 705, and on the DA Form 5500 (Body Fat Assessment Worksheet - Male) or DA Form 5501 (Body Fat Assessment Worksheet - Female) if a body fat assessment is required.

3–5. Enrollment in the Army Body Composition Program

a. Active Army and RC Soldiers who exceed body fat standards in appendix B will be enrolled in the unit ABCP. Enrollment in the ABCP starts on the day that the Soldier is notified by the unit commander (or designee) that he or she has been entered in the program (see para 3–6 for guidance on notification counseling).

b. While enrolled, Soldiers will be provided exercise guidance by the unit master fitness trainer and/or unit fitness training NCO in accordance with FM 7–22; nutrition counseling by registered dietitian (or health care provider, if a dietitian is not available); and assistance in behavioral modification, as appropriate, to help them attain the requirements of the Army.

c. Initial entry Soldiers who exceed body fat standards after 180 days from date of entry to active service will be entered in the ABCP and flagged under the provisions of AR 600–8–2 by the unit commander.

3–6. Actions, counselings, and evaluations for Active Component and Reserve Component Soldiers on active duty

The following actions are required when a Soldier is determined to be exceeding the body fat standard (see table 3–1):

a. Notification counseling. In accordance with AR 600–8–2, the commander has 3 working days to Flag the Soldier using DA Form 268 (Report to Suspend Favorable Personnel Actions (FLAG)) and 2 working days from initiation of DA Form 268 to counsel and/or notify and enroll the Soldier in the ABCP. The effective date of the DA Form 268 flagging action is the date that the Soldier is found to be noncompliant. Notification counseling documentation will be completed in accordance with figure 3–1. During this notification counseling, Soldiers will be advised they—

(1) Have a DA Form 268 placed on their record to suspend favorable personnel actions. Some of the ramifications of the flagging action include:

(a) Are nonpromotable (to the extent such nonpromotion is permitted by law).

(b) Will not be assigned to command, command sergeant major, or first sergeant positions.

(c) In accordance with AR 350–1, are not authorized to attend military schools and institutional training courses.

(2) Are enrolled in the ABCP effective immediately. While enrolled they—

(a) Must read the online U.S. Army Public Health Command (USAPHC) Technical Guide (TG) 358 within 14 days of enrollment and schedule an appointment with a dietitian, if available, or health care provider.

(b) Must complete and return their Soldier Action Plan (refer to para *b*, below) to the commander within 14 days of the notification counseling.

(c) Are required to meet with a dietitian or health care provider within 30 days of enrollment in the ABCP, bring a copy of the commander's request for nutrition counseling (fig 3–2) and their Soldier Action Plan to the dietitian for review, and provide the commander a memorandum signed by the dietitian (or health care provider if a dietitian is not available) verifying that the nutritional counseling took place.

(d) Must participate in unit monthly ABCP assessments to document their progress.

(e) Must meet the body fat standard in order to be released from the ABCP.

(f) Must demonstrate satisfactory progress, as defined in paragraph 3–9*b*, while enrolled in the ABCP and understand that failure to do so will result in bar to reenlistment or initiation of separation proceedings.

(g) May request a medical examination if there is reason to believe that there is an underlying medical condition that may be the direct cause of weight gain or the direct cause of the inability to lose weight or body fat.

(3) Must acknowledge enrollment in the ABCP by memorandum to the commander (see fig 3–3) within 2 working days of notification of enrollment.

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MEMORANDUM FOR (*Soldier's Name, Unit*)

SUBJECT: Army Body Composition Program Enrollment

1. You have been determined to exceed the body fat standard. Effective today you are enrolled in the Army Body Composition Program (ABCP). While enrolled, you will complete the following in accordance with the timeline outlined in AR 600-9, paragraph 3-6 for Active Component and Reserve Component Soldiers on active duty or paragraph 3-7 for Reserve Component Soldiers not on active duty:

a. Read the online USAPHC TG 358 (Army Weight Management Guide) available at http://phc.amedd.army.mil/PHC%20Resource%20Library/USAPHC_TG_358_Army_Weight_Management_Guide.pdf.

b. Complete and submit the Soldier Action Plan within 14 days of enrollment to the commander.

c. (*May*) or (*Must*) meet with a registered dietician within 30 days of enrollment and provide a memorandum from the health care provider stating nutritional counseling took place.

d. Participate in monthly unit body fat assessments.

e. Participate in commanders' and self-directed physical fitness programs within the parameters of any existing temporary or permanent profile.

f. May request a medical examination.

2. You have been flagged under the provisions of AR 600-8-2 and entered in a body composition program. A DA Form 268 (Report to Suspend Favorable Personnel Actions (FLAG)) has been placed in your record. Some ramifications of this flagging action include:

a. You are nonpromotable (to the extent such nonpromotion is permitted by law).

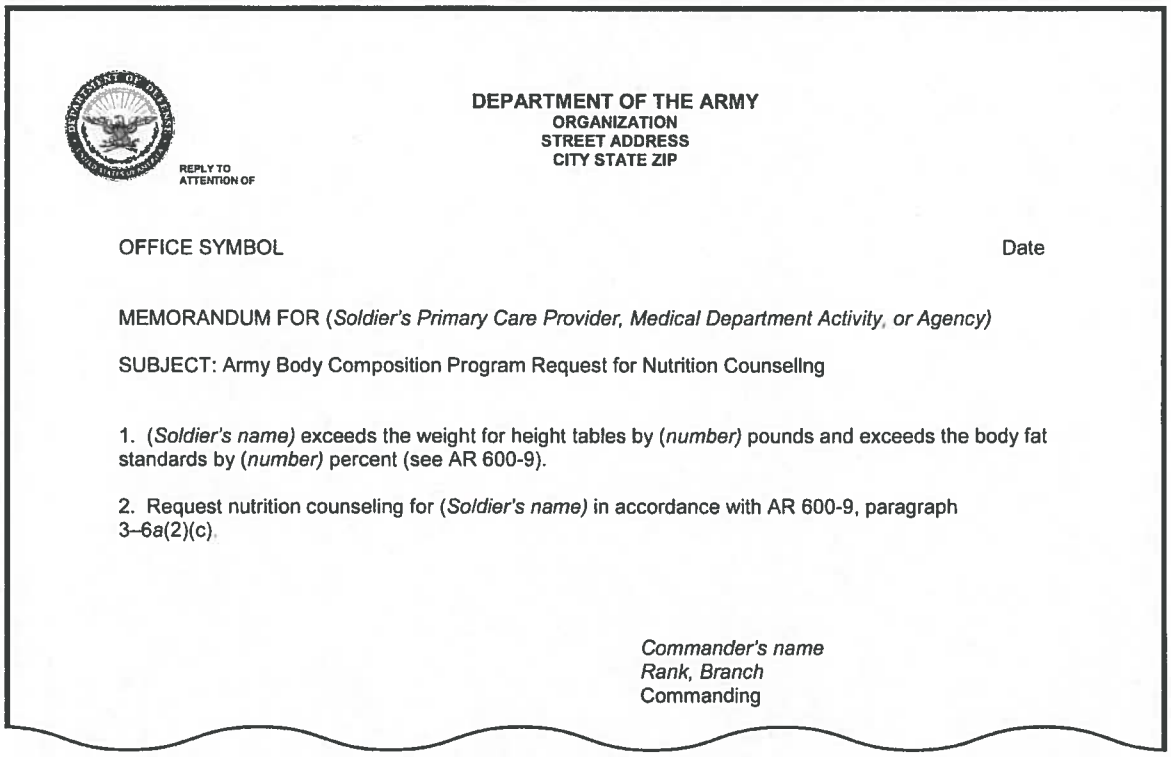
b. You will not be assigned to command, command sergeant major, or first sergeant positions.

c. You are not authorized to attend professional military schools and institutional training courses.

3. A goal of 3 to 8 pounds of weight loss or 1% body fat reduction per month is considered to be satisfactory progress. Failure to make satisfactory progress or achieve the body fat standard will result in a bar from reenlistment or separation from service. You must meet the body fat standard to be released from the ABCP.

Commander's Name
Rank, Branch
Commanding

Figure 3–1. Sample of initial Soldier notification counseling



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MEMORANDUM FOR (*Soldier's Primary Care Provider, Medical Department Activity, or Agency)*

SUBJECT: Army Body Composition Program Request for Nutrition Counselling

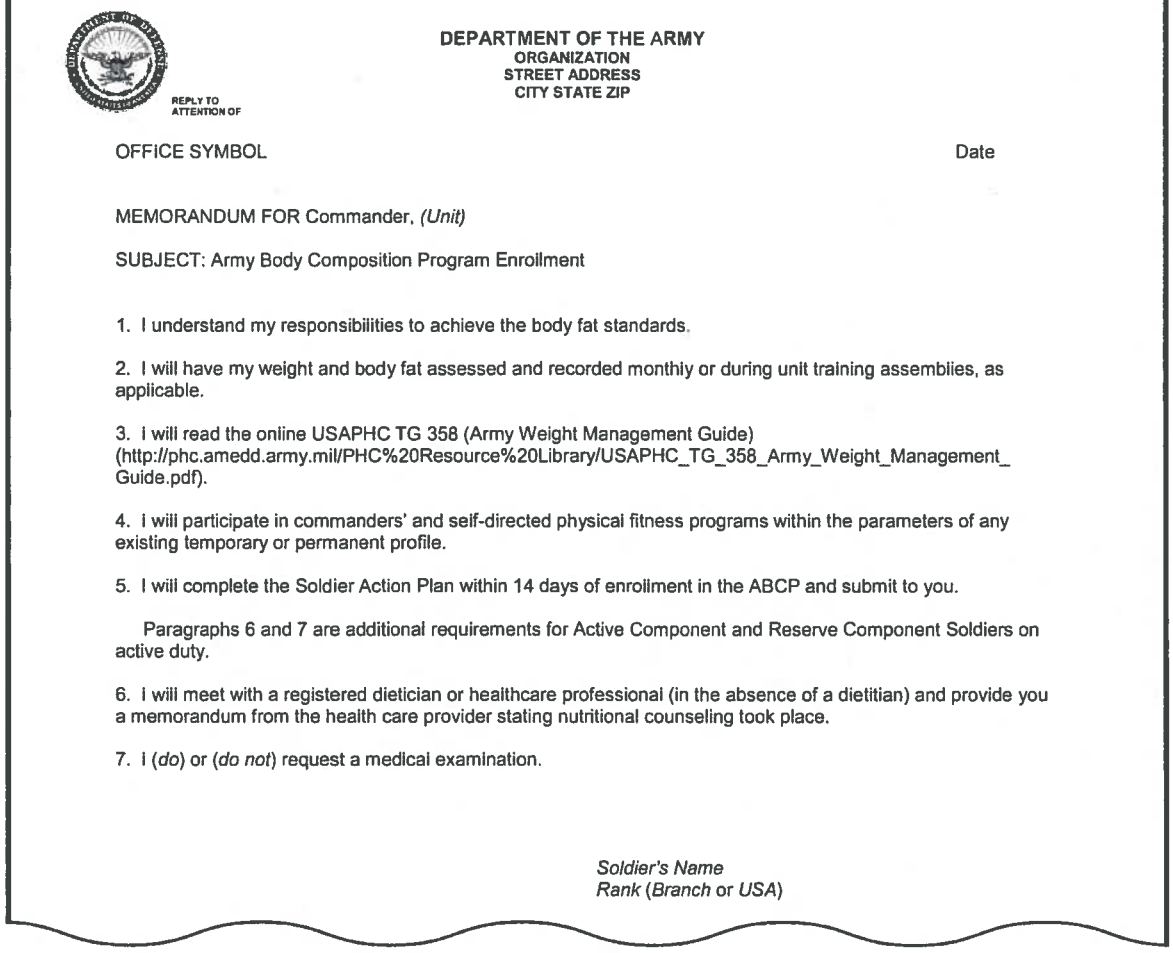
1. (*Soldier's name)* exceeds the weight for height tables by (*number)* pounds and exceeds the body fat standards by (*number)* percent (see AR 600-9).

2. Request nutrition counseling for (*Soldier's name)* in accordance with AR 600-9, paragraph 3–6*a*(2)(c).

Commander's name
Rank, Branch
Commanding

Figure 3–2. Sample of request for nutrition counseling

b. Soldier Action Plan. Within 14 days of the notification counseling, the Soldier will respond to the commander with a Soldier Action Plan confirming that he or she has read USAPHC TG 358, provide date and time of scheduled nutrition counseling, and indicate what approach he or she intends to use to work towards meeting the body fat standard. As a part of the Soldier Action Plan, the Soldier must complete the Army MOVE!23 (http://usaphcapps.amedd.army.mil/move23/register.asp) interactive questionnaire, review the survey results, and record the retrieval code. During the nutrition counseling, the Soldier should provide this retrieval code to the dietitian or health care provider to enable him or her to review the Soldier's responses and provide feedback. The Soldier has the option to modify his or her plan while enrolled in the ABCP (for example, a Soldier may initially opt to follow a commercial weight loss program, but then 2 months later decide to enroll in a no-cost internet-based program). A sample Soldier Action Plan is at figure 3–4.



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MEMORANDUM FOR Commander, *(Unit)*

SUBJECT: Army Body Composition Program Enrollment

1. I understand my responsibilities to achieve the body fat standards.

2. I will have my weight and body fat assessed and recorded monthly or during unit training assemblies, as applicable.

3. I will read the online USAPHC TG 358 (Army Weight Management Guide) (http://phc.amedd.army.mil/PHC%20Resource%20Library/USAPHC_TG_358_Army_Weight_Management_Guide.pdf).

4. I will participate in commanders' and self-directed physical fitness programs within the parameters of any existing temporary or permanent profile.

5. I will complete the Soldier Action Plan within 14 days of enrollment in the ABCP and submit to you.

Paragraphs 6 and 7 are additional requirements for Active Component and Reserve Component Soldiers on active duty.

6. I will meet with a registered dietician or healthcare professional (in the absence of a dietitian) and provide you a memorandum from the health care provider stating nutritional counseling took place.

7. I (*do*) or (*do not*) request a medical examination.

Soldier's Name
Rank (*Branch* or *USA*)

Figure 3–3. Sample of Soldier acknowledgment of enrollment in the Army Body Composition Program

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MEMORANDUM FOR Commander, *(Unit)*

SUBJECT: Soldier Action Plan for the Army Body Composition Program

1. I, *(Soldier's name)*, understand my responsibilities to meet the Army body fat standards and to have my body fat measured and recorded monthly until I meet standards per AR 600-9.

2. I have read USAPHC TG 358 (Army Weight Management Guide) and familiarized myself with the contents. In addition, I understand it is my responsibility to take action and seek out resources to improve my eating choices, as necessary, to assist in meeting Army readiness requirements.

3. I have completed the Army MOVE!23 interactive questionnaire at http://usaphcapps.amedd.army.mil/move23/register.asp. In addition, I reviewed the analysis and recorded my retrieval code for review during my nutrition counseling.

4. I have selected one of the following weight loss or nutrition counseling options as outlined in USAPHC TG 358:

Option A: Weight loss program at the installation medical treatment facility (MTF)
Appointment: *(month/day/year)* at *(time)*

Option B: Registered dietitian visits (if MTF does not have a weight loss program)
Appointment: *(month/day/year)* at *(time)*

Option C: Approved online weight loss program (at own expense)
Name of program: *(program name)*

Option D: Approved commercial weight loss program (at own expense)
Name of program: *(program name)*

Option E: Self-directed program (attach program plan)

Paragraph 5 is an additional requirement for Active Component and Reserve Component Soldiers on active duty only.

5. Per AR 600-9, I have scheduled an appointment with a registered dietitian or health care professional, in the absence of a registered dietitian, at the MTF for nutrition and weight loss education within 30 days of counseling by the commander. I will bring a copy of my Soldier Action Plan to the dietitian appointment for review.
Appointment Date: (*month/day/year*) at (*time*)

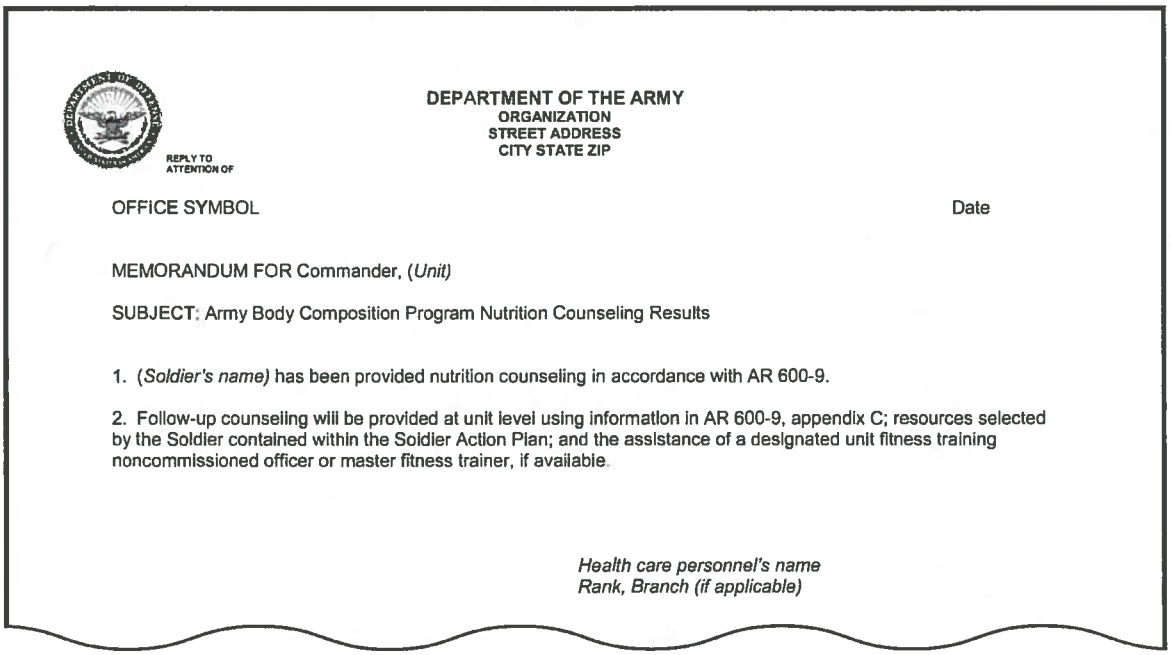
6. By my signature below, I acknowledge, understand, accept, and agree to comply with the information contained in USAPHC TG 358 and as indicated above.

Soldier's signature
Soldier's name
Rank, (*Branch* or *USA*)

Commander's signature
Commander's name
Rank, Branch
Commanding

Figure 3–4. Sample of Soldier Action Plan

c. Nutrition counseling. The Soldier has 30 days after enrollment in the ABCP to meet with a dietitian (or health care provider, if a dietitian is not available) to receive nutrition counseling. Soldiers will schedule this appointment and coordinate any absence with their supervisory chain. Soldiers will provide the commander a memorandum signed by a dietitian or health care provider verifying that the nutrition counseling took place. A sample memorandum is at figure 3–5.



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MEMORANDUM FOR Commander, (*Unit*)

SUBJECT: Army Body Composition Program Nutrition Counseling Results

1. (*Soldier's name*) has been provided nutrition counseling in accordance with AR 600-9.

2. Follow-up counseling will be provided at unit level using information in AR 600-9, appendix C; resources selected by the Soldier contained within the Soldier Action Plan; and the assistance of a designated unit fitness training noncommissioned officer or master fitness trainer, if available.

Health care personnel's name
Rank, Branch (if applicable)

Figure 3–5. Sample of nutrition counseling results

3–7. Actions, counselings, and evaluations for Reserve Component Soldiers not on active duty

The following is required when a Soldier is determined to exceed the body fat standard (see table 3–1):

a. Notification counseling. In accordance with AR 600–8–2, the commander has until the final unit training assembly of that weekend's multiple unit training assembly (MUTA) to Flag the Soldier using DA Form 268. Soldiers will be counseled regarding the initiation of the DA Form 268 prior to the conclusion of the first training period following the date the flagging action was initiated in accordance with AR 600–8–2. The effective date of the flagging action is the date the Soldier is found to be noncompliant. During this notification counseling, Soldiers will be advised they—

(1) Have a DA Form 268 placed on their record to suspend favorable personnel actions. Some of the ramifications of the flagging action include:

(a) Are nonpromotable (to the extent such nonpromotion is permitted by law).

(b) Will not be assigned to command, command sergeant major, or first sergeant positions.

(c) In accordance with AR 350–1, are not authorized to attend military schools and institutional training courses.

(2) Are enrolled in the ABCP effective immediately. While enrolled they—

(a) Must read the USAPHC TG 358 within 14 days of enrollment. An appointment with a dietitian is optional at the Soldier's own expense.

(b) Must complete and return their Soldier Action Plan (refer to para *b*, below) to the commander prior to the conclusion of the first training period after being notified of enrollment in the ABCP.

(c) Must participate in unit monthly ABCP assessments to document their progress.

(d) Must meet the body fat standard in order to be released from the ABCP.

(e) Must demonstrate satisfactory progress, as defined in paragraph 3–9*b*, while enrolled in the ABCP and understand that failure to do so will result in bar to reenlistment, initiation of separation proceedings, or a transfer into the IRR.

(f) May request a medical examination if there is reason to believe that there is an underlying medical condition that may directly contribute to weight gain or prevent weight or body fat loss. This exam is at the Soldier's own expense.

(3) Must acknowledge enrollment in the ABCP by memorandum to the commander (see fig 3–3) no later than the following MUTA after the notification of enrollment.

b. Soldier Action Plan. At the next scheduled MUTA following ABCP enrollment notification counseling, Soldiers will respond to the commander with a Soldier Action Plan confirming that they have read USAPHC TG 358. As a part of the Soldier Action Plan, Soldiers must complete the Army MOVE!23 (http://usaphcapps.amedd.army.mil/move23/register.asp) interactive questionnaire, review the survey results, and record their retrieval code. The retrieval code is to be recorded in the event the Soldiers choose to review the results with a dietitian or health care provider during a nutrition counseling appointment. Soldiers have the option to modify their plan while enrolled in the ABCP (for example, a Soldier may initially opt to follow a commercial weight loss program, but then 2 months later decide to enroll in a no-cost internet-based program). A sample Soldier Action Plan is at figure 3–4.

c. Nutrition counseling. This is optional at the Soldier's own expense.

Table 3–1
Summary of Army Body Composition Program-related actions, counseling, and evaluations

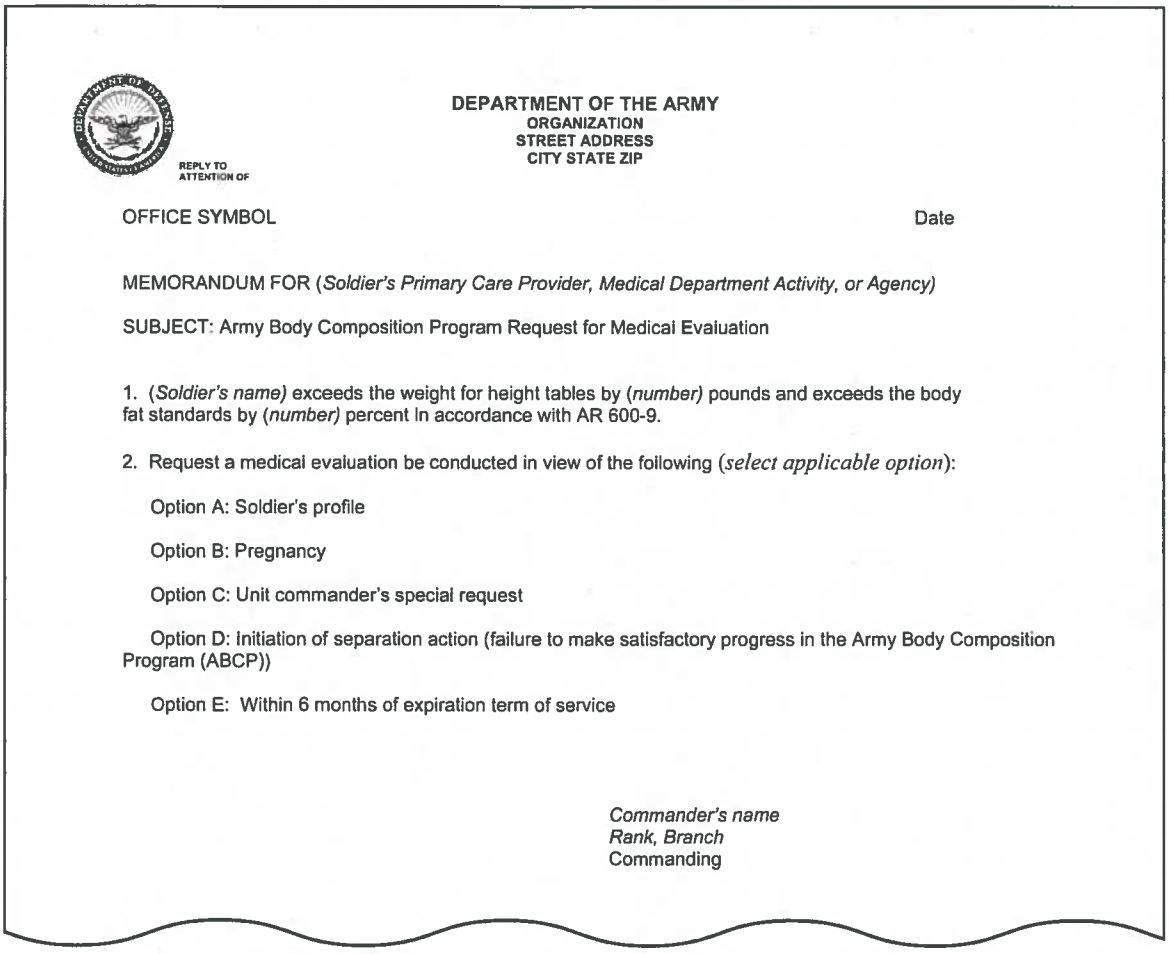
Action, counseling, and/or evaluation	Who	Requirement	Timing	
			AC and RC on active duty	RC not on active duty
Flagging action (DA Form 268)	Commander	Mandatory	3 working days (after Soldier determined to exceed body fat standard)	Before end of MUTA in which Soldier is determined to exceed body fat
Notification counseling	Commander	Mandatory	2 working days from when DA Form 268 is initiated	No later than the next MUTA after Soldier is determined to exceed body fat
Soldier acknowledgment in ABCP	Soldier	Mandatory	2 working days (after Soldier receives notification counseling)	No later than the next MUTA after the notification counseling
Read USAPHC TG 358 and complete Army MOVE!23 Questionnaire	Soldier	Mandatory	14 days (after Soldier receives notification counseling)	14 days (after Soldier receives notification counseling)
Soldier weight and body fat assessment	Commander/ Designee	Mandatory	Monthly	Monthly
Soldier Action Plan	Soldier	Mandatory	14 days (after Soldier receives notification counseling)	No later than the next MUTA after the notification counseling
Nutrition counseling memorandum	Dietitian	Mandatory (AC and RC on active duty only)	Within first 30 days (after Soldier receives notification counseling)	Not applicable
Medical evaluation memorandum	Medical professional	Optional	Upon enrollment in ABCP (Soldier or commander may request it)	Upon enrollment in ABCP (Soldier may request it) at Soldier's own expense
		Mandatory (AC only)	Soldier is pregnant Prior to bar to reenlistment or separation actions (commander must request it)	Soldier is pregnant (provides documentation from health care provider)

3–8. Administrative requirements

Commanders must maintain an ABCP file at the unit on each Soldier enrolled in the program. Each file must include, at a minimum, the following for each enrollment:

a. DA Form 268 initiating the flagging action.
b. DA Form 5500 or DA Form 5501 from enrollment and each monthly assessment.
c. Notification counseling (see fig 3–1).
d. Soldier Action Plan (see fig 3–4).
e. Nutrition counseling results memorandum (AC and RC on active duty only) (see fig 3–5).
f. Medical evaluation request memorandum(s), if indicated (AC and RC on active duty only) (see fig 3–6).
g. Medical evaluation results, if indicated (AC and RC on active duty only) (see fig 3–7).

h. Release from ABCP counseling memorandum from the unit commander (see fig 3–8).
i. Copy of DA Form 3349 (Physical Profile), if indicated.



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MEMORANDUM FOR (*Soldier's Primary Care Provider, Medical Department Activity, or Agency)*

SUBJECT: Army Body Composition Program Request for Medical Evaluation

1. (*Soldier's name)* exceeds the weight for height tables by (*number)* pounds and exceeds the body fat standards by (*number)* percent In accordance with AR 600-9.

2. Request a medical evaluation be conducted in view of the following (*select applicable option*):

Option A: Soldier's profile

Option B: Pregnancy

Option C: Unit commander's special request

Option D: Initiation of separation action (failure to make satisfactory progress in the Army Body Composition Program (ABCP))

Option E: Within 6 months of expiration term of service

Commander's name
Rank, Branch
Commanding

Figure 3–6. Sample of request for medical evaluation

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MEMORANDUM FOR Commander, *(Unit)*

SUBJECT: Army Body Composition Program Medical Evaluation Results

1. This memorandum is to provide information concerning the evaluation of (*Soldier's name)* in accordance with AR 600-9.

2. Based on my examination and evaluation, the Soldier listed above is (*select applicable option*):

Option A: Medically cleared to fully participate in the Army Body Composition Program (ABCP).

Option B: Not medically cleared to participate in the ABCP.

3. If not medically cleared to fully participate in the ABCP, the following applies (*select applicable option*):

Option A: The Soldier is pregnant and is temporarily exempt from the requirements of the ABCP, in accordance with AR 600-9, paragraph 3-15.

Option B: The Soldier has an underlying temporary medical condition that directly causes weight gain and/or prevents weight loss, which requires treatment. The Soldier can participate in the ABCP but should not be penalized (processed for separation/bar) if unable to show progress. The estimated time before the Soldier can fully participate in the ABCP is *(specify number, not to exceed 6)* months, in accordance with AR 600-9, paragraph 3-11.

Option C: The Soldier has a permanent medical condition that requires referral to a medical evaluation board or physical evaluation board. The Soldier can participate in the ABCP as required, however, he or she will not be penalized (processed for separation/bar) if unable to show progress. If the board results determine Soldier is fit for duty (retained) and the Soldier is still not in compliance with AR 600-9, the Soldier will be fully enrolled in the ABCP and required to show satisfactory progress.

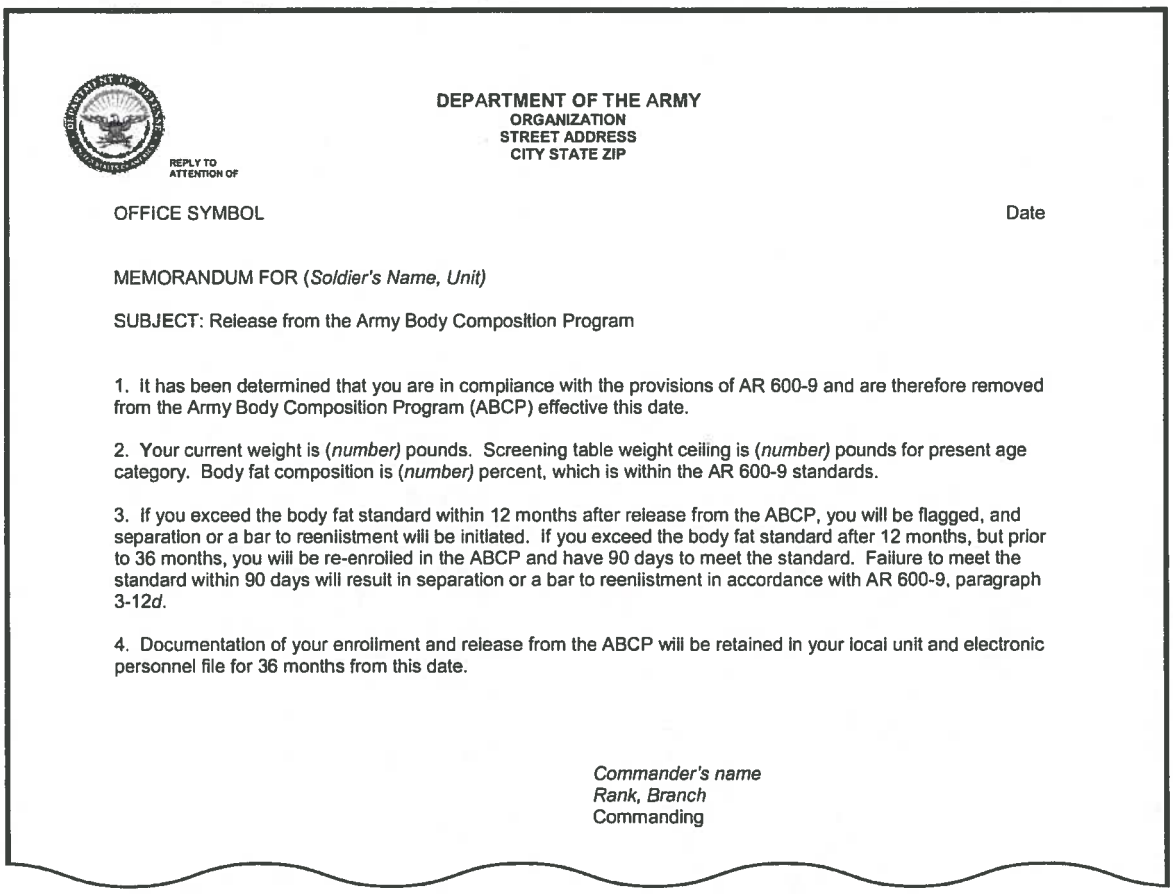
4. If medically cleared, the Soldier will be enrolled in or continued in the ABCP and administratively handled in accordance with AR 600-9.

5. The Soldier may participate in unit physical training (*fully*) or (*within exercise restrictions outlined in the Soldier's temporary or permanent Physical Profile (DA Form 3349)*).

6. Point of contact for this memorandum is the undersigned at (*email address and phone number*).

Health care personnel's name
Rank, Branch (if applicable)

Figure 3–7. Sample of medical evaluation results



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MEMORANDUM FOR (*Soldier's Name, Unit)*

SUBJECT: Release from the Army Body Composition Program

1. It has been determined that you are in compliance with the provisions of AR 600-9 and are therefore removed from the Army Body Composition Program (ABCP) effective this date.

2. Your current weight is (*number)* pounds. Screening table weight ceiling is (*number)* pounds for present age category. Body fat composition is (*number)* percent, which is within the AR 600-9 standards.

3. If you exceed the body fat standard within 12 months after release from the ABCP, you will be flagged, and separation or a bar to reenlistment will be initiated. If you exceed the body fat standard after 12 months, but prior to 36 months, you will be re-enrolled in the ABCP and have 90 days to meet the standard. Failure to meet the standard within 90 days will result in separation or a bar to reenlistment in accordance with AR 600-9, paragraph 3-12*d*.

4. Documentation of your enrollment and release from the ABCP will be retained in your local unit and electronic personnel file for 36 months from this date.

Commander's name
Rank, Branch
Commanding

Figure 3–8. Sample of release from the Army Body Composition Program

3–9. Monitoring Soldier progress in the Army Body Composition Program

a. Approximately every 30 days (or during unit assemblies for RC not on active duty), commanders will conduct a monthly ABCP assessment to measure Soldier progress, with results annotated on DA Form 5500 or DA Form 5501. During monthly assessments, every Soldier enrolled in the ABCP will be weighed and have a body fat assessment conducted in order to document weight and fat loss progress.

b. A monthly loss of either 3 to 8 pounds or 1 percent body fat are both considered to be safely attainable goals that enable Soldiers to lose excess body fat and meet the body fat standards. Soldiers that meet either of these goals are considered to be making satisfactory progress in the ABCP.

c. When necessary, commanders and supervisors will provide additional support, guidance, and resources to enhance Soldier's success. This may include time to participate in ongoing nutritional counseling or weight loss programs as prescribed by the dietitian or health care provider. Helpful tips for commanders and supervisors are located in appendix C.

3–10. Medical evaluation

a. A medical evaluation is required when:

(1) Requested by the unit commander.

(2) Requested by the Soldier (at own expense for RC Soldier not on active duty).

(3) Soldier is being considered for separation for failure to make satisfactory progress in the ABCP (applies to AC and RC on active duty only).

(4) Soldier is within 6 months of expiration term of service after the initiation of a reenlistment bar for failure to make satisfactory progress in the ABCP.

b. The health care provider will conduct a medical evaluation to ensure the Soldier can participate in the ABCP and

rule out any underlying medical condition that may be a direct cause of significant weight gain or directly inhibit weight or body fat loss. If an underlying medical condition is found, the following applies:

(1) If the medical condition is temporary and can be controlled with medication or other medical treatment and meets the retention standards of AR 40–501, the health care provider will—

(a) Initiate treatment.

(b) In accordance with AR 40–501, prepare a temporary profile in the e-Profile application within the Medical Operational Data System (MODS) (https://apps.mods.army.mil) listing any functional limitations that would prevent the Soldier from fully participating in the ABCP.

(c) Complete the memorandum (fig 3–7) and return to the commander for enrollment in the ABCP.

(d) Refer to appropriate specialist for nutritional and exercise counseling.

(e) RC personnel not on active duty may choose to self-refer to their personal physician (at their own expense) for further evaluation or treatment.

(2) If the medical condition does not meet medical retention standards of AR 40–501 (see medical fitness standards for retention and separation, including retirement) the health care provider will refer the Soldier to a medical evaluation board.

c. Aircraft crewmembers exceeding the body fat standards will be referred to a flight surgeon for medical evaluation and determination of impact on flight status.

d. Health care providers will not use the e-Profile application within the MODS (https://apps.mods.army.mil) to recommend exemption from ABCP for temporary medical conditions. Health care providers will use the medical evaluation results memorandum (fig 3–7) for this purpose.

3–11. Temporary medical condition

a. All Soldiers found to exceed the allowable body fat standard will have a DA Form 268 initiated and be enrolled in the ABCP.

b. Soldiers found to have a temporary medical condition that directly causes weight gain or prevents weight or body fat loss will have up to 6 months from the initial medical evaluation date to undergo treatment to resolve the medical condition. The medical specialty physician may extend the time period up to 12 months if it is determined more time is needed to resolve the medical condition. During this time, the Soldier will participate in the ABCP, to include initiation of a DA Form 268, nutrition counseling, and monthly body fat assessment, but will not be penalized for failing to show progress. However, if the Soldier meets the body fat standard during this timeframe, he or she will be removed from the ABCP.

c. The provisions of this paragraph are not applicable to medical conditions or injuries based solely on a prescribed reduction in physical activity. The inability to exercise does not directly cause weight gain. Health care personnel will advise Soldiers to modify caloric intake when reduced physical activity is necessary as part of a treatment plan.

d. Once the medical condition is resolved, or 6 months (not to exceed 12 months), whichever occurs first, from the date of the medical evaluation, and if the Soldier still exceeds the body fat standard, he or she will continue participating in the ABCP but will be required to show satisfactory progress, as defined in paragraph 3–9*b*. Health care providers will forward to the Soldier's commander an updated memorandum stating the effective date that the Soldier's temporary medical condition is resolved.

e. If the Soldier is unable to show satisfactory progress in accordance with paragraph 3–9*b*, the Soldier will be subject to separation.

3–12. Program failure

a. Satisfactory progress in the ABCP is defined as a monthly weight loss of either 3 to 8 pounds or 1 percent body fat.

b. A Soldier enrolled in the ABCP is considered to be failing the program if:

(1) He or she exhibits less than satisfactory progress on two consecutive monthly ABCP assessments; or

(2) After 6 months in the ABCP he or she still exceeds body fat standards, and exhibits less than satisfactory progress for three or more (nonconsecutive) monthly ABCP assessments.

c. When a Soldier has failed the program, the commander will request a medical evaluation.

(1) If the medical evaluation finds the Soldier has a medical condition that does not meet medical retention standards of AR 40–501 (see medical fitness standards for retention and separation, including retirement) the Soldier will be processed in accordance with AR 40–501 (see chap 3, disposition).

(2) If the Soldier is found to have a temporary underlying medical condition that directly causes weight gain or prevents weight or body fat loss, the commander will follow the requirement in paragraph 3–11*b*.

(3) If the medical evaluation finds no underlying medical condition, then the commander will initiate separation action, bar to reenlistment, or involuntary transfer to the IRR for RC Soldiers in accordance with AR 140–10.

(4) For RC personnel not on active duty only, if the individual has not obtained an evaluation from his or her personal physician under the provisions of paragraph 3–7*a*(2)(f) and cannot demonstrate that the overweight condition

results from an underlying or associated disease process, the individual may be separated under appropriate regulations without further medical evaluation by health care personnel.

d. The commander or supervisor will inform the Soldier, in writing, that a bar to reenlistment, separation action, or a transfer to the IRR is being initiated under the following applicable regulation(s): AR 135–175; AR 135–178; AR 600–8–24 (see eliminations and miscellaneous types of separations); AR 601–280; AR 635–200; AR 140–10; National Guard Regulation (NGR) (AR) 600–5; NGR 600–101; NGR 600–200; or NGR 635–100.

3–13. Release from the Army Body Composition Program

a. Commanders and supervisors will remove individuals administratively from the ABCP as soon as the body fat standard is achieved. Soldiers that meet the screening table weight must remain in the ABCP program until they no longer exceed the required body fat standard.

b. The commander will remove the DA Form 268 actions and counsel the Soldier on the importance of maintaining body composition and potential consequences if re-enrolled in the program within 36 months. A sample memorandum of release from ABCP counseling is at figure 3–8.

3–14. Body fat assessment failure within 36 months of release from Army Body Composition Program

a. If a Soldier again exceeds the body fat standard within 12 months after release from the ABCP, a DA Form 268 will be initiated on the Soldier. The Soldier will undergo a medical evaluation (at own expense for RC not on active duty).

(1) If the Soldier is found to have a temporary medical condition that prevents weight or body fat loss, the commander will follow the requirements of paragraph 3–11.

(2) If no underlying medical condition is found, the commander will initiate separation action, bar to reenlistment, or transfer to the IRR per paragraph 3–12*d*.

b. If, after 12 months but less than 36 months from the date of release from the ABCP, it is determined that a Soldier again exceeds the body fat standard, a DA Form 268 will be initiated on the Soldier. The Soldier will undergo a medical evaluation (at own expense for RC not on active duty).

(1) If the Soldier is found to have a temporary medical condition that prevents weight or body fat loss, the commander will re-enroll the Soldier in the ABCP under the requirements of paragraph 3–11.

(2) If no underlying medical condition is found, the commander will re-enroll the Soldier in the ABCP. The Soldier will have 90 days to meet the standards. Soldiers who meet the body fat standard at the 90-day point will be released from the ABCP. Soldiers who do not meet the ABCP body fat standard at the 90-day point are considered ABCP failures. Commanders will initiate separation action, bar to reenlistment, or transfer to the IRR per paragraph 3–12*d* for all Soldiers who fail to meet the body fat standard at the 90-day point.

3–15. Pregnancy

a. Personnel who meet this regulation's standards and become pregnant will be exempt from the standards for the duration of the pregnancy plus the period of 180 days after the pregnancy ends. If, after this period of exemption they are verified to exceed the body fat standard, they will be enrolled in the ABCP, pending approval of a medical doctor that they are fit to participate in the program.

b. Soldiers who become pregnant while enrolled in the ABCP will remain under the flagging action.

c. Soldiers entered or re-entered in the ABCP after pregnancy will be considered first-time entries into the program; paragraph 3–14 will not apply at that time.

d. If the Soldier is determined to exceed the body fat standard and is identified to have a temporary underlying medical condition, refer to paragraph 3–11 for appropriate actions.

3–16. Hospitalization

Personnel who meet this regulation's standards and are hospitalized for 30 continuous days or more will be exempt from the standards for the duration of the hospitalization and the recovery period as specified by their profile, not to exceed 90 days from discharge from the hospital. If at the end of the specified recovery period the Soldier exceeds the allowable body fat standard, a DA Form 268 will be initiated on the Soldier and he or she will be enrolled in the ABCP.

3–17. Exception to policy authority

a. The DCS, G–1 is the approval authority for all exceptions to this regulation. All requests for an exception to this policy will include an endorsement from a medical professional and be processed through the Soldier's chain of command, with recommendations as to disposition from the company, battalion, and brigade-level commanders, reviewed by the servicing staff judge advocate, and submitted directly to Deputy Chief of Staff, G–1 (DAPE–HR), 300 Army Pentagon, Washington, DC 20310–0300 for final determination.

b. The use of certain medications to treat an underlying medical or psychological disorder or the inability to perform

all aerobic events may contribute to weight gain but are not considered sufficient justification for noncompliance with this regulation. Medical professionals should advise Soldiers taking medications that may contribute to weight gain, or Soldiers with temporary or permanent physical profiles, that they are still required to meet the body fat standard established in the regulation; the Soldier may be referred to an appropriate specialist for nutrition and exercise counseling as indicated.

c. Chronic medical conditions will not be used to exempt Soldiers from meeting the standards established in this regulation.

d. There are no exemptions to the provisions of this regulation based solely on race, ethnicity, or gender.

3–18. Reenlistment criteria

a. Personnel who exceed the body fat standard in appendix B will not be allowed to reenlist or extend their enlistment.

b. Exceptions to policy for Active Army personnel (including RC personnel on active duty) are prescribed in this subparagraph. For Soldiers who are otherwise physically fit and have performed their duties in a satisfactory manner, the commander exercising General Court Martial Convening Authority or the first general officer in the Soldier's normal chain of command (whichever is in the most direct line to the Soldier) may approve the following exceptions to policy:

(1) Extension of enlistment may be authorized for personnel who meet one of the following criteria:

(a) Individuals who have a temporary medical condition that directly precludes loss of weight or body fat. In such cases, the type of ongoing treatment will be documented and the extension will be for the minimum time necessary to correct the condition and achieve the required weight or body fat loss.

(b) Pregnant Soldiers (except those Soldiers who have medical conditions as listed in para 3–15*d*) who are otherwise fully qualified for reenlistment, including those with approved exception to policy, but who exceed acceptable standards prescribed in this regulation, will be extended for the minimum period that will allow birth of the child, plus 7 months. A clearance from the doctor that the Soldier is medically fit to participate in the ABCP is required. Authority, which will be cited on DA Form 1695 (Oath of Extension of Enlistment) is AR 601–280 (see determination of qualifications). On completion of the period of extension, the Soldier will be reevaluated under paragraph 3–15.

(2) Exceptions to policy allowing reenlistment and/or extension of enlistment are authorized only in cases where medically documented conditions (see para 3–11) preclude attainment of required standards.

c. All requests for extension of enlistment for ARNG and USAR (troop program unit and IRR) personnel not on active duty will be processed under NGR 600–200 or AR 140–111 (see extending enlistment or reenlistment agreements), as appropriate.

d. Requests for exceptions to policy will be forwarded through the chain of command, with the commander's personal recommendation and appropriate comment at each level. As a minimum, requests will include:

(1) The physician's evaluation.

(2) A record of progress in the ABCP.

(3) Current height and weight.

(4) Current body fat assessment results.

(5) Years of active Federal service.

(6) Other pertinent information.

e. Soldiers who have completed a minimum of 18 years of active Federal service may, if otherwise eligible, be extended for the minimum time required to complete 20 years active Federal service. Retirement must be accomplished no later than the last day of the month in which the Soldier attains retirement eligibility. Application for retirement will be submitted at the time extension is authorized. Approval and/or disapproval authority is outlined in AR 601–280.

f. USAR Soldiers who have completed a minimum of 18 years of qualifying service for retired pay at age 60 may be extended for the minimum time required to complete 20 years qualifying service. Approval and/or disapproval authority is outlined in AR 140–111. Transfer to the IRR or Retired Reserve or discharge will be accomplished at the end of the retirement year in which the Soldier attains the 20 qualifying years.

g. ARNG Soldiers who have completed a minimum of 18 years qualifying service for retired pay at age 60 may be extended for the minimum time required to complete 20 years qualifying service by the State Adjutant General; disapproval authority is the Secretary of the Army. Transfer to the IRR or Retired Reserve or discharge will be accomplished at the end of the retired year in which the Soldier attains the 20 qualifying years.

Appendix A
References

Section I
Required Publications

Army regulations are available online from the Army Publishing Directorate Web site at http://www.apd.army.mil/.

AR 135–175
Separation of Officers (Cited in para 3–12*d*.)

AR 135–178
Enlisted Administrative Separations (Cited in para 3–12*d*.)

AR 140–10
Assignments, Attachments, Details, and Transfers (Cited in paras 3–12*c*(3), 3–12*d*.)

AR 140–111
U.S. Army Reserve Reenlistment Program (Cited in paras 3–18*c*, 3–18*f*.)

AR 600–8–2
Suspension of Favorable Personnel Actions (Flag) (Cited in paras 3–2*e*, 3–3*b*(5), 3–5*c*, 3–6*a*, 3–7*a*.)

AR 600–8–24
Officer Transfers and Discharges (Cited in para 3–12*d*.)

AR 601–280
Army Retention Program (Cited in paras 3–12*d*, 3–18*b*(1)(b), 3–18*e*.)

AR 635–40
Physical Evaluation for Retention, Retirement, or Separation (Cited in para 3–3*b*(2).)

AR 635–200
Active Duty Enlisted Administrative Separations (Cited in para 3–12*d*.)

AR 670–1
Wear and Appearance of Army Uniforms and Insignia (Cited in para 1–5*b*(1)(d).)

DODI 1308.3
DOD Physical Fitness and Body Fat Programs Procedures (Cited on title page (summary).) (Available at http://www.dtic.mil/whs/directives.)

NGR (AR) 600–5
The Active Guard/Reserve (AGR) Program, Title 32, Full-Time National Guard Duty (FTNGD) (Cited in para 3–12*d*.) (Available at http://www.ngbpdc.ngb.army.mil/.)

NGR 600–101
Warrant Officers-Federal Recognition and Related Personnel Actions (Cited in para 3–12*d*.) (Available at http://www.ngbpdc.ngb.army.mil/.)

NGR 600–200
Enlisted Personnel Management (Cited in paras 3–12*d*, 3–18*c*.) (Available at http://www.ngbpdc.ngb.army.mil/.)

NGR 635–100
Termination of Appointment and Withdrawal of Federal Recognition (Cited in para 3–12*d*.) (Available at http://www.ngbpdc.ngb.army.mil/.)

FM 7–22
Army Physical Readiness Training (Cited in paras 2–18, 3–5*b*.) (Available at http://armypubs.army.mil/doctrine/Active_FM.html.)

USAPHC TG 358
Army Weight Management Guide (Cited in paras 3–6*a*(2)(a), 3–6*b*, 3–7*a*(2)(a) and *b*, C–7, figs 3–1, 3–3, 3–4.) (Available at http://phc.amedd.army.mil/PHC%20Resource%20Library/USAPHC_TG_358_Army_Weight_Management_Guide.pdf.)

Section II
Related Publications
A related publication is a source of additional information. The user does not have to read a related publication to understand this publication.

AR 11–2
Managers' Internal Control Program

AR 25–30
The Army Publishing Program

AR 40–25
Nutrition Standards and Education

AR 40–501
Standards of Medical Fitness

AR 350–1
Army Training and Leader Development

Section III
Prescribed Forms
Unless otherwise indicated, DA forms are available on the Army Publishing Directorate Web site at www.apd.army.mil.

DA Form 5500
Body Fat Assessment Worksheet (Male) (Prescribed in paras 3–4*d*, 3–8*b*, 3–9*a*, B–1*b*, B–6, table B–3.)

DA Form 5501
Body Fat Assessment Worksheet (Female) (Prescribed in paras 3–4*d*, 3–8*b*, 3–9*a*, B–1*b*, B–6, table B–4.)

Section IV
Referenced Forms

DA Form 11–2
Internal Control Evaluation Certification

DA Form 268
Report to Suspend Favorable Personnel Actions (FLAG)

DA Form 705
Army Physical Fitness Test Scorecard

DA Form 1695
Oath of Extension of Enlistment

DA Form 2028
Recommended Changes to Publications and Blank Forms

DA Form 3349
Physical Profile

Appendix B
Standard Methods for Determining Body Fat Using Body Circumferences, Height, and Weight

B–1. Introduction

a. The procedures for the measurements of height, weight, and specific body circumferences for the estimation of body fat are described in this appendix. The weight for height table is listed in table B–1 followed by the body fat standards in table B–2.

b. Although circumferences may be looked upon by untrained personnel as easy measures, they can give erroneous results if proper technique is not followed. The individual taking the measurements must have a thorough understanding of the appropriate body landmarks and measurement techniques. Unit commanders will require that designated personnel have read the instructions regarding technique and location and obtained adequate practice before official body fat determinations are made. Individuals taking the measurements will be designated unit fitness trainers, certified master fitness trainers, and/or trained in body circumference methodology, as specified in para 2–18*c*. Two members of the unit will be utilized in the taking of measurements; one to place the tape measure and determine measurements and the other to assure proper placement and tension of the tape, as well as to record the measurement on the worksheet (DA Form 5500 and DA Form 5501). Soldiers should be measured by trained individuals of the same gender. If a trained individual of the same gender is not available to conduct the measurements, a female Soldier will be present when a male measures a female, and a male Soldier will be present when a female measures a male. The two will work with the Soldier between them so the tape is clearly visible from all sides. Take all circumference measurements sequentially three times and record them to the nearest half inch. If any one of the three closest measurements differs by more than 1 inch from the other two, take an additional measurement and compute a mathematical average of the three measurements with the least difference to the nearest half inch and record this value.

c. Soldiers will be measured for body fat in stocking feet and standard Army physical fitness uniform trunks and T-shirt. Undergarments that may serve to bind the abdomen, hip, or thigh areas are not authorized for wear when a Soldier is being measured for body fat composition. This includes, but is not limited to spandex shorts or girdle-like undergarments.

d. When measuring circumferences, compression of the soft tissue requires constant attention. The tape will be applied so it makes contact with the skin and conforms to the body surface being measured. It will not compress the underlying soft tissues. However, the hip circumference measurement requires more firm pressure to compress the authorized physical fitness uniform trunks. All measurements are made in the horizontal plane (parallel to the floor), unless indicated otherwise.

e. The tape measure will be made of a nonstretchable material, preferably fiberglass; cloth or steel tapes are unacceptable. Cloth measuring tapes will stretch with usage and most steel tapes do not conform to body surfaces. The tape measure will be calibrated, that is, compared with a yardstick or a metal ruler to ensure validity. This is done by aligning the fiberglass tape measure with the quarter-inch markings on the ruler. The markings will match those on the ruler; if not, do not use that tape measure. The tape will be one-quarter to one-half inch wide (not exceeding one-half inch) and a minimum of 5 feet in length. A retractable fiberglass tape is the best type for measuring all areas.

Note. Tapes are currently available through the Army Supply System (Federal stock number 5210–01–238–8103 or national stock number 8315–01–238–8103). The current Army supply system or any other fiberglass tape (not to exceed one-half inch) may be used if retractable tapes cannot be purchased by unit budget funds available and if approved by installation commanders.

B–2. Height and weight measurements

a. The height will be measured with the Soldier in stocking feet (without running shoes) and wearing the authorized physical fitness uniform (trunks and T-shirt). The Soldier will stand on a flat surface with the head held horizontal, looking directly forward with the line of vision horizontal and the chin parallel to the floor. The body will be straight but not rigid, similar to the position of attention. When measuring height to determine body fat percentage (fig B–1 or B–2), the Soldier's height is measured to the nearest half inch. When measuring height to use the weight for height screening table (table B–1) the Soldier's height is measured and then rounded to the nearest inch with the following guidelines:

(1) If the height fraction is less than half an inch, round down to the nearest whole number in inches.

(2) If the height fraction is half an inch or greater, round up to the next highest whole number in inches.

b. The weight will be measured with the Soldier in stocking feet and wearing the authorized physical fitness uniform (trunks and T-shirt); running shoes will not be worn. Scales used for weight measurement will be calibrated annually for accuracy. The measurement will be made on scales available in units and recorded to the nearest pound with the following guidelines:

(1) If the weight fraction of the Soldier is less than one-half pound, round down to the nearest pound.

(2) If the weight fraction of the Soldier is one half-pound or greater, round up to the next whole pound.

(3) No weight will be deducted to account for clothing.

Table B–1
Weight for height table (screening table weight)

		Male weight in pounds, by age				Female weight in pounds, by age			
Height (inches)	Minimum weight[1] (pounds)	17–20	21–27	28–39	40+	17–20	21–27	28–39	40+
58	91	-	-	-	-	119	121	122	124
59	94	-	-	-	-	124	125	126	128
60	97	132	136	139	141	128	129	131	133
61	100	136	140	144	146	132	134	135	137
62	104	141	144	148	150	136	138	140	142
63	107	145	149	153	155	141	143	144	146
64	110	150	154	158	160	145	147	149	151
65	114	155	159	163	165	150	152	154	156
66	117	160	163	168	170	155	156	158	161
67	121	165	169	174	176	159	161	163	166
68	125	170	174	179	181	164	166	168	171
69	128	175	179	184	186	169	171	173	176
70	132	180	185	189	192	174	176	178	181
71	136	185	189	194	197	179	181	183	186
72	140	190	195	200	203	184	186	188	191
73	144	195	200	205	208	189	191	194	197
74	148	201	206	211	214	194	197	199	202
75	152	206	212	217	220	200	202	204	208
76	156	212	217	223	226	205	207	210	213
77	160	218	223	229	232	210	213	215	219
78	164	223	229	235	238	216	218	221	225
79	168	229	235	241	244	221	224	227	230
80[2]	173	234	240	247	250	227	230	233	236

Notes:
[1] Male and female Soldiers who fall below the minimum weights shown in table B–1 will be referred by the commander for immediate medical evaluation.
[2] Add 6 pounds per inch for males over 80 inches and 5 pounds per inch for females over 80 inches.

Table B–2
Maximum allowable percent body fat standards

Age group: 17–20
Male (% body fat): 20%
Female (% body fat): 30%

Age group: 21–27
Male (% body fat): 22%
Female (% body fat): 32%

Age group: 28–39
Male (% body fat): 24%
Female (% body fat): 34%

Age group: 40 and older
Male (% body fat): 26%
Female (% body fat): 36%

Circumference Value	Height (inches)																		
	60	60.5	61	61.5	62	62.5	63	63.5	64	64.5	65	65.5	66	66.5	67	67.5	68	68.5	69
13.5	9	9																	
14	11	11	10	10	10	10	9	9											
14.5	12	12	12	11	11	11	11	10	10	10	10	9	9						
15	13	13	13	13	12	12	12	12	11	11	11	11	10	10	10	10	10	9	9
15.5	15	15	15	15	15	13	13	13	13	12	12	12	12	11	11	11	11	11	10
16	16	16	15	15	15	15	14	14	14	14	13	13	13	13	12	12	12	12	12
16.5	17	17	16	16	16	16	15	15	15	15	14	14	14	14	14	13	13	13	13
17	18	18	18	17	17	17	17	16	16	16	16	15	15	15	15	14	14	14	14
17.5	19	19	19	18	18	18	18	17	17	17	17	16	16	16	16	16	15	15	15
18	20	20	20	19	19	19	19	18	18	18	18	18	17	17	17	17	16	16	16
18.5	21	21	21	20	20	20	20	19	19	19	19	19	18	18	18	18	17	17	17
19	22	22	22	21	21	21	21	20	20	20	20	20	19	19	19	19	18	18	18
19.5	23	23	23	22	22	22	22	21	21	21	21	21	20	20	20	20	19	19	19
20	24	24	24	23	23	23	23	22	22	22	22	21	21	21	21	21	20	20	20
20.5	25	25	25	24	24	24	24	23	23	23	23	22	22	22	22	21	21	21	21
21	26	26	26	25	25	25	25	24	24	24	24	23	23	23	23	22	22	22	22
21.5	27	27	27	26	26	26	26	25	25	25	24	24	24	24	23	23	23	23	23
22	28	27	27	27	27	26	28	26	28	25	25	25	25	25	24	24	24	24	23
22.5	29	28	28	28	28	27	27	27	27	26	26	26	26	25	25	25	25	24	24
23	29	29	29	29	28	28	28	28	27	27	27	27	26	26	26	26	26	25	25
23.5	30	30	30	29	29	29	29	28	28	28	28	27	27	27	27	27	26	26	26
24	31	31	30	30	30	30	29	29	29	29	28	28	28	28	28	27	27	27	27
24.5	32	31	31	31	30	30	30	30	30	29	29	29	29	29	28	28	28	28	27
25	32	32	32	32	31	31	31	31	30	30	30	30	30	29	29	29	29	28	28
25.5	33	33	33	32	32	32	32	31	31	31	31	31	30	30	30	30	29	29	29
26	34	34	33	33	33	33	32	32	32	32	31	31	31	31	31	30	30	30	30
26.5	35	34	34	34	34	33	33	33	33	32	32	32	32	32	31	31	31	31	30
27	35	35	35	35	34	34	34	34	33	33	33	33	32	32	32	32	32	31	31
27.5	36	36	36	35	35	35	35	34	34	34	34	33	33	33	33	32	32	32	32
28	37	36	36	36	36	35	35	35	35	34	34	34	34	34	33	33	33	33	32
28.5			37	37	36	36	36	36	35	35	35	35	34	34	34	34	34	33	33
29					37	37	37	36	36	36	36	38	36	36	35	35	35	35	34
29.5								37	37	36	36	36	36	36	35	35	35	35	34
30											37	37	36	36	36	36	35	35	35
30.5													37	37	37	36	36	36	36
31																37	37	36	36
31.5																			37
32																			
32.5																			
33																			
33.5																			
34																			
34.5																			
35																			

Figure B–1. Percent fat estimates for males

Circumference Value	Height (inches)																				
	69.5	70	70.5	71	71.5	72	72.5	73	73.5	74	74.5	75	75.5	76	76.5	77	77.5	78	78.5	79	79.5
13.5																					
14																					
14.5																					
15																					
15.5	10	10	10	9	9	9															
16	11	11	11	11	10	10	10	10	10	9	9										
16.5	12	12	12	12	12	11	11	11	11	11	10	10	10	10	10	9	9				
17	14	13	13	13	13	13	12	12	12	12	11	11	11	11	11	10	10	10	10	10	9
17.5	15	14	14	14	14	14	13	13	13	13	13	12	12	12	12	12	11	11	11	11	11
18	16	15	15	15	15	15	14	14	14	14	14	13	13	13	13	13	12	12	12	12	12
18.5	17	17	16	16	16	16	15	15	15	15	15	14	14	14	14	14	13	13	13	13	13
19	18	18	17	17	17	17	16	16	16	16	16	15	15	15	15	15	14	14	14	14	14
19.5	19	18	18	18	18	18	17	17	17	17	17	16	1	16	16	16	15	15	15	15	15
20	20	19	19	19	19	19	18	18	18	18	18	17	17	17	17	17	16	16	16	16	16
20.5	21	20	20	20	20	19	19	19	19	19	18	18	18	18	18	17	17	17	17	17	16
21	21	21	21	21	21	20	20	20	20	20	19	19	19	19	19	18	18	18	18	18	17
21.5	22	22	22	22	21	21	21	21	21	20	20	20	20	20	19	19	19	19	19	18	18
22	23	23	23	23	22	22	22	22	22	21	21	21	21	20	20	20	20	20	20	19	19
22.5	24	24	24	23	23	23	23	23	22	22	22	21	21	20	20	20	20	20	20	19	19
23	25	25	24	24	24	24	24	23	23	23	23	23	22	22	22	22	22	21	21	21	21
23.5	26	25	25	25	25	25	24	24	24	24	24	23	23	23	23	23	22	22	22	22	22
24	26	26	26	26	26	25	25	25	25	25	24	24	24	24	24	23	23	23	23	23	22
24.5	27	27	27	27	26	26	26	26	26	25	25	25	25	25	24	24	24	24	24	23	23
25	28	28	28	27	27	27	27	26	26	26	26	26	25	25	25	25	25	24	24	24	24
25.5	29	29	28	28	28	28	27	27	27	27	27	26	26	26	26	26	25	25	25	25	25
26	29	29	29	29	29	28	28	28	28	28	27	27	27	27	27	26	26	26	26	26	25
26.5	30	30	30	30	29	29	29	29	28	28	28	28	28	27	27	27	27	27	26	26	26
27	31	31	30	30	30	30	30	29	29	29	29	29	28	28	28	28	28	27	27	27	27
27.5	32	31	31	31	31	30	30	30	30	30	29	29	29	29	29	28	28	28	28	28	27
28	32	32	32	32	31	31	31	31	31	30	30	30	30	29	29	29	29	29	29	28	28
28.5	33	33	32	32	32	32	32	31	31	31	31	31	30	30	30	30	30	29	29	29	29
29	34	33	33	33	33	32	32	32	32	32	31	31	31	31	31	30	30	30	30	30	29
29.5	34	34	34	34	33	33	33	33	32	32	32	32	32	31	31	31	31	31	30	30	30
30	35	35	34	34	34	34	34	33	33	33	33	32	32	32	32	32	31	31	31	31	31
30.5	35	35	35	35	35	34	34	34	34	34	33	33	33	33	32	32	32	32	32	32	31
31	36	36	36	35	35	35	35	35	34	34	34	34	33	33	33	33	33	33	32	32	32
31.5	37	36	36	36	36	36	35	35	35	35	35	34	34	34	34	33	33	33	33	33	33
32		37	37	37	36	36	36	36	36	35	35	35	35	34	34	34	34	34	33	33	33
32.5					37	37	36	36	36	36	36	35	35	35	35	35	34	34	34	34	34
33								37	37	36	36	36	36	36	35	35	35	35	35	34	34
33.5											37	37	36	36	36	36	36	35	35	35	35
34													37	37	37	36	36	36	36	36	35
34.5																37	37	37	36	36	38
35																			37	37	36

Figure B–1. Percent fat estimates for males-Continued

Circumference Value	Height (inches)																			
	58	58.5	59	59.5	60	60.5	61	61.5	62	62.5	63	63.5	64	64.5	65	65.5	66	66.5	67	67.5
45	19																			
45.5	20	20	19																	
46	21	20	20	20	19															
46.5	21	21	21	20	20	20	19	19												
47	22	22	22	21	21	20	20	20	19	19										
47.5	23	23	22	22	22	21	21	21	20	20	19	19								
48	24	23	23	23	22	22	22	21	21	21	20	20	20	19						
48.5	24	24	24	23	23	23	22	22	22	21	21	21	20	20	20	19				
49	25	25	24	24	24	23	23	23	22	22	22	21	21	21	20	20	20	19	19	
49.5	26	26	25	25	24	24	24	23	23	23	22	22	22	21	21	21	20	20	20	19
50	27	26	26	26	25	25	24	24	24	23	23	23	22	22	22	22	21	21	21	20
50.5	27	27	27	26	26	26	25	25	25	24	24	23	23	23	23	22	22	22	21	21
51	28	28	27	27	27	26	26	26	25	25	25	24	24	24	23	23	23	22	22	22
51.5	29	28	28	28	27	27	27	26	26	26	25	25	25	24	24	24	23	23	23	22
52	29	29	29	28	28	28	27	27	27	26	26	26	25	25	25	24	24	24	23	23
52.5	30	30	29	29	29	28	28	28	27	27	27	26	26	26	25	25	25	24	24	24
53	31	30	30	30	29	29	29	28	28	28	27	27	27	26	26	26	25	25	25	24
53.5	31	31	31	30	30	30	29	29	29	28	28	28	27	27	27	26	26	26	25	25
54	32	32	31	31	31	30	30	30	29	29	29	28	28	28	27	27	27	26	26	26
54.5	33	32	32	32	31	31	31	30	30	30	29	29	29	28	28	28	27	27	27	26
55	33	33	33	32	32	32	31	31	31	30	30	30	29	29	29	28	28	28	27	27
55.5	34	34	33	33	33	32	32	32	31	31	31	30	30	30	29	29	29	28	28	28
56	35	34	34	34	33	33	33	32	32	31	31	31	30	30	30	30	29	29	29	28
56.5	35	35	35	34	34	34	33	33	32	32	32	31	31	31	30	30	30	29	29	29
57	36	36	35	35	34	34	34	33	33	33	32	32	32	31	31	31	30	30	30	29
57.5	37	36	36	35	35	35	34	34	34	33	33	33	32	32	32	31	31	31	30	30
58	37	37	36	36	36	35	35	35	34	34	34	33	33	33	32	32	32	31	31	31
58.5	38	37	37	37	36	36	36	35	35	35	34	34	34	33	33	33	32	32	32	31
59	38	38	38	37	37	37	36	36	36	35	35	35	34	34	34	33	33	33	32	32
59.5	39	39	38	38	38	37	37	36	36	36	35	35	35	34	34	34	33	33	33	33
60	40	39	39	38	38	38	37	37	37	36	36	36	35	35	35	34	34	34	33	33
60.5	40	40	39	39	39	38	38	38	37	37	37	36	36	36	35	35	35	34	34	34
61	41	40	40	40	39	39	39	38	38	38	37	37	37	36	35	36	35	35	35	34
61.5	41	41	41	40	40	40	39	39	38	38	38	37	37	37	36	36	36	36	35	35
62	42	42	41	41	40	40	40	39	39	39	38	38	38	37	37	37	36	36	36	35
62.5	42	42	42	41	41	41	40	40	40	39	39	39	38	38	38	37	37	37	36	36
63	43	43	42	42	42	41	41	41	40	40	40	39	39	39	38	38	38	37	37	37
63.5	44	43	43	42	42	42	41	41	41	40	40	40	39	39	39	38	38	38	37	37
64	44	44	43	43	43	42	42	42	41	41	41	40	40	40	39	39	39	38	38	38
64.5	45	44	44	44	43	43	43	42	42	42	41	41	41	40	40	40	39	39	39	38
65	45	45	45	44	44	43	43	43	42	42	42	41	41	41	40	40	40	39	39	39
65.5	46	45	45	45	44	44	44	43	43	43	42	42	42	41	41	41	40	40	40	39
66	46	46	46	45	45	45	44	44	43	43	43	42	42	42	41	41	41	41	40	40
66.5	47	46	46	46	45	45	45	44	44	44	43	43	43	42	42	42	41	41	41	40
67			47	46	46	46	45	45	45	44	44	44	43	43	43	42	42	42	41	41
67.5				47	46	46	46	45	45	45	44	44	44	43	43	43	42	42	42	41
68					47	47	46	46	46	45	45	45	44	44	44	43	43	43	42	42
68.5							47	46	46	46	45	45	45	44	44	44	43	43	43	43
69								47	47	46	46	46	45	45	45	44	44	44	43	43
69.5										47	46	46	46	45	45	45	44	44	44	44
70											47	47	46	46	46	45	45	45	44	44
70.5													47	46	46	46	46	45	45	45
71														47	47	46	46	46	45	45
71.5																47	47	46	46	46
72																	47	47	46	46
72.5																			47	47
73																				
73.5																				
74																				
74.5																				
75																				
75.5																				
76																				
76.5																				
77																				
77.5																				
78																				
78.5																				
79																				

Figure B–2. Percent fat estimates for females

Circumference Value	Height (inches)													
	66	66.5	67	67.5	68	68.5	69	69.5	70	70.5	71	71.5	72	72.5
45														
45.5														
46														
46.5														
47														
47.5														
48														
48.5														
49	20	19	19											
49.5	20	20	20	19	19									
50	21	21	21	20	20	20	19							
50.5	22	22	21	21	21	20	20	20	19	19				
51	23	22	22	22	22	22	21	21	21	20	20	20	20	19
51.5	23	23	23	22	22	22	21	21	21	20	20	20	20	19
52	24	24	23	23	23	22	22	22	21	21	21	21	20	20
52.5	25	24	24	24	23	23	23	22	22	22	22	21	21	21
53	25	25	25	24	24	24	23	23	23	22	22	22	22	21
53.5	26	26	25	25	25	24	24	24	23	23	23	23	22	22
54	27	26	26	26	25	25	25	24	24	24	24	23	23	23
54.5	27	27	27	26	26	26	25	25	25	24	24	24	24	23
55	28	28	27	27	27	26	26	26	25	25	25	25	24	24
55.5	29	28	28	28	27	27	27	26	26	26	25	25	25	25
56	29	29	29	28	28	28	27	27	27	26	26	26	25	25
56.5	30	29	29	29	29	28	28	28	27	27	27	26	26	26
57	30	30	30	29	29	29	29	28	28	28	27	27	27	28
57.5	31	31	30	30	30	29	29	29	29	28	28	28	27	27
58	32	31	31	31	30	30	30	29	29	29	29	28	28	28
58.5	32	32	32	31	31	31	30	30	30	29	29	29	29	28
59	33	33	32	32	32	31	31	31	30	30	30	29	29	29
59.5	33	33	33	33	32	32	32	31	31	31	30	30	30	29
60	34	34	33	33	33	32	32	32	32	31	31	31	30	30
60.5	35	34	34	34	33	33	33	32	32	32	32	31	31	31
61	35	35	35	34	34	34	33	33	33	32	32	32	32	31
61.5	36	36	35	35	35	34	34	34	33	33	33	32	32	32
62	36	36	36	35	35	35	35	34	34	34	33	33	33	32
62.5	37	37	36	36	36	35	35	35	34	34	34	34	33	33
63	38	37	37	37	36	36	36	35	35	35	34	34	34	34
63.5	38	38	37	37	37	37	36	36	36	35	35	35	34	34
64	39	38	38	38	37	37	37	36	36	36	36	35	35	35
64.5	39	39	39	38	38	38	37	37	37	36	36	36	36	35
65	40	39	39	39	38	38	38	38	37	37	37	36	36	36
65.5	40	40	40	39	39	39	38	38	38	37	37	37	37	36
66	41	41	40	40	40	39	39	39	38	38	38	37	37	37
66.5	41	41	41	40	40	40	39	39	39	39	38	38	38	37
67	42	42	41	41	41	40	40	40	39	39	39	39	38	38
67.5	42	42	42	41	41	41	41	40	40	40	39	39	39	38
68	43	43	42	42	42	41	41	41	40	40	40	40	39	39
68.5	43	43	43	43	42	42	42	41	41	41	40	40	40	39
69	44	44	43	43	43	42	42	42	41	41	41	41	40	40
69.5	44	44	44	44	43	43	43	42	42	42	41	41	41	41
70	45	45	44	44	44	43	43	43	43	42	42	42	41	41
70.5	46	45	45	45	44	44	44	43	43	43	42	42	42	42
71	46	46	45	45	45	44	44	44	44	43	43	43	42	42
71.5	47	46	46	46	45	45	45	44	44	44	43	43	43	43
72	47	47	46	46	46	45	45	45	45	44	44	44	43	43
72.5			47	47	46	46	46	45	45	45	44	44	44	44
73					47	46	46	46	45	45	45	45	44	44
73.5						47	47	46	46	46	45	45	45	44
74								47	46	46	46	46	45	45
74.5									47	47	46	46	46	45
75											47	46	46	46
75.5												47	47	46
76														47

Figure B–2. Percent fat estimates for females-Continued

B–3. Description of circumference sites and their anatomical landmarks and technique

a. All circumference measurements will be taken three times and recorded to the nearest half inch (or 0.50). Each sequential measurement should be within 1 inch of the next or previous measurement. If the measurements are within 1 inch of each other, derive a mathematical average to the nearest half of an inch. If any one of the three measurements differs by more than 1 inch, take an additional measurement. Then, average the three closest measures.

b. Each set of measurements will be completed sequentially to discourage assumption of repeated measurement readings. For males, complete one set of neck and abdomen measurements, not three neck circumferences followed by three abdomen circumferences. Continue the process by measuring the neck and abdomen in series until three sets of measurements have been completed. For females, complete one set of neck, waist (abdomen), and hip measurements, not three neck circumferences followed by three waist (abdomen) circumferences, and so on. Continue the process by measuring neck, waist (abdomen), and hip series until three sets of measurements have been completed.

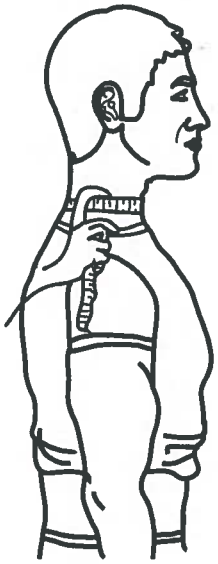
c. Instructions for computing body fat are at tables B–3 (males) and B–4 (females). Percent fat estimates are shown in figures B–1 (males) and B–2 (females). Illustrations of each tape measurement are at figures B–3 (males) and B–4 (females).

Table B–3
Instructions for completing DA Form 5500 (male)

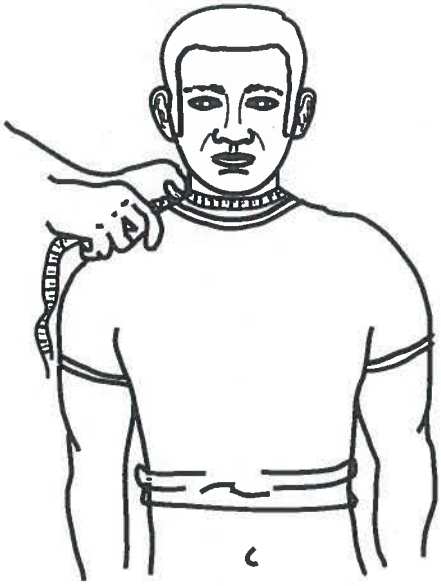
NAME	Print the Soldier's last name, first name, and middle initial in NAME block.
RANK	Print rank in the RANK box.
HEIGHT	Measure the Soldier's height as described in this appendix to the nearest half inch and record the measurement in HEIGHT block.
WEIGHT	Measure the Soldier's weight as described in this appendix to the nearest pound and record in WEIGHT block.
	Note: Follow the rounding rules for rounding height and weight measurement as described earlier in this appendix.
AGE	Print age in years in AGE block.
STEP 1	Neck measurement. Measure Soldier's neck circumference at a point just below the larynx (Adam's apple and perpendicular to the long axis of the neck). The Soldier should look straight ahead during the measurement, with shoulders down (not hunched). Round the neck measurement up to nearest half inch and record in block labeled FIRST.
STEP 2	Abdominal measurement. Measure the Soldier's abdominal circumference to nearest half inch. Round down to nearest half inch and record in block labeled FIRST.
	Note: Repeat STEPS 1 and 2 in series until you have completed three sets of neck and abdomen circumference measurements.
STEP 3	Average neck measurement. Find mathematical average of FIRST, SECOND, and THIRD neck circumference by adding them together and dividing by three. Place this number to nearest half inch in block marked AVERAGE for STEPS 1 and 3.
STEP 4	Average abdominal measurement. Find mathematical average of FIRST, SECOND, and THIRD abdominal circumference by adding them together and dividing by three. Place this number to nearest half inch in block marked AVERAGE for STEPS 2 and 4.
STEP 5	Circumference value equals abdominal circumference (STEP 4) minus neck circumference (STEP 3). Subtract STEP 4 from STEP 3 and enter results in STEP 5.
STEP 6	Height factor. Enter the height in inches to the nearest half inch.
	Note: Follow the rules for rounding of height and weight measurements as described earlier in this appendix.
STEP 7	Percent body fat. Determine percent body fat by finding Soldier's circumference value (value listed in STEP 5) and height in inches (value listed in STEP 6) in figure B–1. The percent body fat is the value that intercepts with circumference value and height in inches as listed in figure B–1. This is the Soldier's PERCENT BODY FAT.
	Note: Go to figure B–1 to locate the circumference value (abdomen minus neck difference) in the left-hand column.

Table B–4
Instructions for completing DA Form 5501 (female)

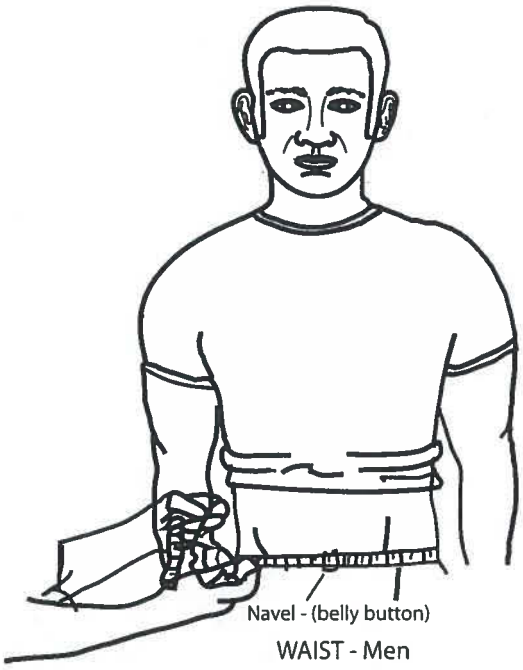
NAME	Print Soldier's last name, first name, and middle initial in NAME block.
RANK	Print rank in RANK block.
HEIGHT	Measure Soldier's height as described in this appendix to nearest half inch and record the measurement in HEIGHT block.
WEIGHT	Measure Soldier's weight as described in this appendix to nearest pound and record in WEIGHT block.
	Note: Follow the rules for rounding of height and weight measurement as described earlier in this appendix.
AGE	Print age in years in AGE block.
STEP 1	Neck measurement. Measure Soldier's neck circumference at a point just below the larynx (Adam's apple and perpendicular to the long axis of the neck). The Soldier should look straight ahead during the measurement, with shoulders down (not hunched). Round the neck measurement up to nearest half inch and record in block labeled FIRST.
STEP 2	Waist (abdomen) measurement. Measure Soldier's natural waist circumference against the skin at the point of minimal abdominal circumference, usually located about halfway between the navel and lower end of sternum (breastbone). If site is not easily visible, take several measurements at probable sites and use the smallest value. Ensure tape is level and parallel to floor. Soldier's arms must be at the sides. Take measurements at the end of Soldier's normal relaxed exhalation. Round the natural waist measurement down to nearest half inch and record in block labeled FIRST.
STEP 3	Hip measurement. Measure Soldier's hip circumference while facing Soldier's right side by placing the tape around the hips so that it passes over the greatest protrusion of the gluteal muscles (buttocks) as viewed from the side. Ensure tape is level and parallel to floor. Apply sufficient tension on tape to minimize effect of clothing. Round hip measurement down to nearest half inch and record in block labeled FIRST.
	Repeat STEPS 1, 2, and 3 in series until you have completed three sets of neck, waist (abdomen), and hip circumference measurements. Find mathematical average of FIRST, SECOND, and THIRD circumference in STEPS 1, 2, and 3 by adding them together and dividing by three for each step. Place this number to nearest half inch in block marked AVERAGE for each step.
STEP 4	Calculations.
Line A	Waist (abdomen) circumference. Enter value from STEP 2 in line 4A.
Line B	Hip circumference. Enter value from STEP 3 in line 4B.
Line C	Total (4A+4B=4C). Add waist circumference (line 4A) and hip circumference (line 4B). Enter result in line 4C.
Line D	Neck circumference. Enter value from STEP 1 in line 4D.
Line E	Circumference value (4C-4D=4E). Subtract value in line 4C from value in line 4D. Enter result in line 4E.
Line F	Enter the height in inches to the nearest half inch in line 4F.
	Note: Follow the rules for rounding of height and weight measurements as described earlier in this appendix.
Line G	Percent body fat. Determine percent body fat by finding Soldier's circumference value (value listed in line 4E) and height in inches (line 4F) in figure B–2. Percent body fat is the value that intercepts with circumference value and height in inches as listed in figure B–2. This is the Soldier's PERCENT BODY FAT.
	Note: Go to figure B–2 to locate the circumference value in the left-hand column.



NECK - Men

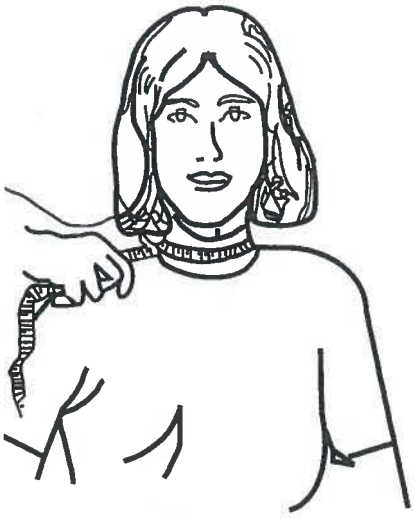


NECK - Men

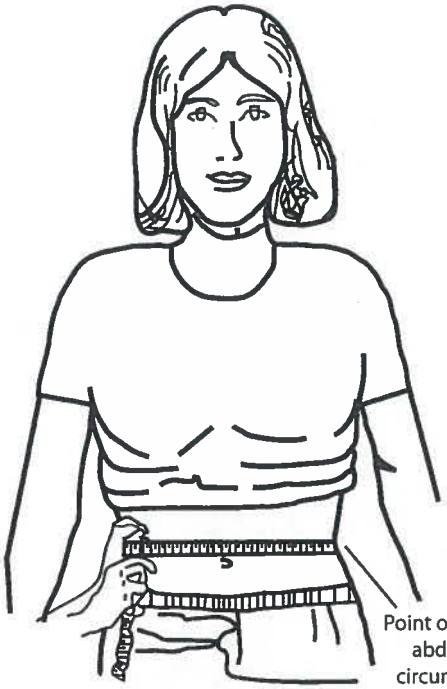


WAIST - Men

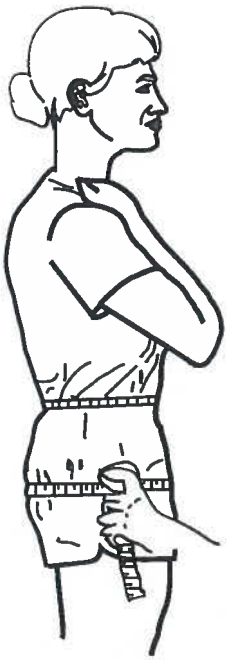
Figure B–3. Male tape measurement illustration



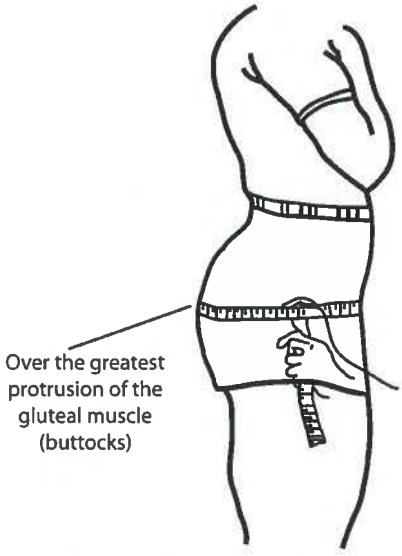
NECK - Women



WAIST - Women



HIP - Women
side measurement



HIP - Women
side measurement

Figure B–4. Female tape measurement illustration

B–4. Circumference sites and landmarks for males

a. Neck. Measure the neck circumference at a point just below the larynx (Adam's apple) and perpendicular to the long axis of the neck. Do not place the tape measure over the Adam's apple. Soldier will look straight ahead during measurement, with shoulders down (not hunched). The tape will be as close to horizontal as anatomically feasible (the tape line in the front of the neck will be at the same height as the tape line in the back of the neck). Care will be taken to ensure the shoulder/neck muscles (trapezius) are not involved in the measurement. Round neck measurement up to the nearest half inch and record (for example, round "16 ¼ inches" to "16 ½ inches").

b. Abdomen. Measure abdominal circumference against the skin at the navel (belly button) level and parallel to the floor. Arms are at the sides. Record the measurement at the end of Soldier's normal, relaxed exhalation. Round abdominal measurement down to the nearest half inch and record (for example, round "34 3/4 inches" to "34 1/2 inches").

B–5. Circumference sites and landmarks for females

a. Neck. This procedure is the same as for males.

b. Waist (abdomen). Measure the natural waist circumference, against the skin, at the point of minimal abdominal circumference. The waist circumference is taken at the narrowest point of the abdomen, usually about halfway between the navel and the end of the sternum (breastbone). When this site is not easily observed, take several measurements at probable sites and record the smallest value. The Soldier's arms must be at the sides. Take measurements at the end of Soldier's normal relaxed exhalation. Tape measurements of the waist will be made directly against the skin. Round the natural waist measurement down to the nearest half inch and record (for example, round "28 5/8 inches" to "28 1/2 inches").

c. Hip. The Soldier taking the measurement will view the person being measured from the side. Place the tape around the hips so that it passes over the greatest protrusion of the gluteal muscles (buttocks), keeping the tape in a horizontal plane (parallel to the floor). Check front to back and side to side to be sure the tape is level to the floor on all sides before the measurements are recorded. Because the Soldier will be wearing authorized physical fitness uniform trunks, the tape can be drawn snugly without compressing the underlying soft tissue to minimize the influence of the shorts on the size of the measurement. Round the hip measurement down to the nearest half inch and record (for example, round "44 3/8 inches" to "44 inches").

B–6. Preparation of DA Form 5500 and DA Form 5501

It is extremely important that the following instructions are read before attempting to complete DA Form 5500 and/or DA Form 5501. Have a copy of the form available when reading these instructions.

a. Tables B–3 and B–4 and figures B–1 through B–4 will provide information needed to prepare DA Form 5500 and DA Form 5501. The instructions for the forms are written in a stepwise fashion. The measurements and computation processes are different for males and females.

b. A DA Form 5500 (male) or DA Form 5501 (female) must be completed for Soldiers who exceed the weight for height table (table B–1) or when a unit commander or supervisor determines that the individual's appearance suggests that body fat is excessive (see para 3–2*d*). The purpose of this form is to help determine the Soldier's percent body fat using the circumference technique described in this regulation.

c. Before starting, have a thorough understanding of the measurements to be made as outlined in this appendix. A scale for measuring body weight, a device for measuring height, and a measuring tape (see specifications in para B–1*d*) for the circumference measurements are also required.

d. If any of the measurements are not listed in figure B–1 or B–2, see table B–5 for guidance on how to calculate body fat percentage.

Note. A scientific calculator, which can be found on computers, must be used. On the computer, pull up 'calculator' from 'programs' and then click on 'view' and choose 'scientific'. Commanders are responsible for the accuracy of all calculations. Use of auto calculators is not authorized.

Note. All measurements must be in inches. Use normal rounding rules for all measurements and calculations unless otherwise specified.

Table B–5
Sample body fat calculations

SAMPLE (WOMEN)
Measurements: Neck=15 inches; Waist=42 inches; Hip=44 inches; Height=64 inches

The equation for women is:
% body fat=[163.205 x Log10 (waist + hip – neck)] – [97.684 x Log10 (height)] – 78.387

A. Solve: [163.205 x Log10 (71)]. Take the Log10 (71)=1.85 (when using a calculator, be careful not to use ln (natural log). Instead, enter 71 and press the LOG key.

B. Solve: [97.684 x Log10 (64)]. Take the Log10 (64)=1.81 (when using a calculator, be careful not to use the ln (natural log). Instead, enter 64 and press the LOG key.

C. Solve the equation:
% body fat=(163.205 x 1.85) – (97.684 x 1.81) – 78.387
=301.93 – 176.81 – 78.387
=47% (actual number is 46.73%; round to the nearest whole %)

SAMPLE (MEN)
Measurements: Neck=16 inches; Waist=49 inches; Height=69 inches

The equation for men is:
% body fat=[86.010 x Log10 (waist – neck)] – [70.041 x Log10 (height)] + 36.76

A. Solve: [86.010 x Log10 (33)]. Take the Log10 (33)=1.52 (when using a calculator, be careful not to use ln (natural log). Instead, enter 33 and press the LOG key.

B. Solve: [70.041 x Log10 (69)]. Take the Log10 (69)=1.84 (when using a calculator, be careful not to use the ln (natural log). Instead, enter 69 and press the LOG key.

C. Solve the equation:
% body fat=(86.010 x 1.521) – (70.041 x 1.841) + 36.76
=130.74 – 128.88 + 36.76
=39% (actual number is 38.62%; round to the nearest whole %)

Appendix C
Weight Loss

C–1. General

Overweight and obesity are significant military medical concerns because these conditions are associated with decreased operational effectiveness. In order to meet Army body fat standards and avoid losing their careers, Soldiers may resort to dangerous tactics. This limits the body's ability to function effectively and hinders physical and cognitive performance. While some weight loss diets may be harmless, others could result in adverse effects that may compromise the health of the Soldier. These diets usually fail in the end and may start a vicious cycle of weight loss and weight regain.

C–2. Leader responsibilities

Leaders must be aware of unsafe weight loss strategies and pay attention to clues that a Soldier might be engaged in unhealthy weight loss practices. Soldiers suspected of engaging in harmful weight loss practices should be referred by the commander to their primary care manager for a medical evaluation. A consultation with a registered dietitian, who can provide guidance in starting a safe and effective weight loss program, is also recommended.

C–3. Key components of a weight loss program

A healthful and safe weight loss program includes these key components:

a. Nutrition therapy.

(1) A reduction of 500 calories per day from the current level will allow for a weight loss of 1 pound per week; a weight loss of no more than 1 to 2 pounds per week is recommended. The best weight loss plan will not be too difficult to follow. It will also help an individual obtain and maintain his or her ideal weight and body fat in the recommended ranges and develop and/or maintain lean muscle tissue required for physical demands.

(2) A healthful diet contains sensible portions of fruits, vegetables, grains, lean protein, and skim and/or low-fat dairy products. In addition, it is recommended that foods and beverages consumed contain little or no added sugar, sodium, and solid fats. Eating four to six small meals per day and not skipping meals, especially breakfast, is helpful for weight loss.

b. Increased physical activity. Physical activity should include aerobic activity, muscular strength and endurance, and flexibility activities. Recommendations:

(1) To maintain a healthy weight: 30 minutes of physical activity 5 to 7 times a week. Bottom line up front: Stay active for a lifetime to keep weight off.

(2) Active weight loss: 60 to 90 minutes of physical activity daily may be needed for weight loss. Physical activity will enhance weight loss as long as the daily resting energy needs are met.

(3) Weight loss maintenance: 30 to 60 minutes daily may be needed to prevent weight gain. Physical activity is the best predictor of weight loss maintenance.

c. Behavior modification. Behavior change is the key to long-term weight management. Specific strategies to change behavior such as self-monitoring, stress management, problem solving, planning, and preparing are needed for successful weight loss and maintenance.

C–4. Unsafe weight loss strategies

a. Fasting or starvation. Crash dieting, fasting, or starvation reduces weight, but also slows down the body's metabolism and forces the body to utilize lean muscle or organs for energy. Prolonged fasting can lead to decrease in muscle endurance and loss of strength and power. Coupled with fluid restriction, the dangers of dehydration are also a factor.

b. Water loss or forced dehydration. Since the body is 75 percent water, this is the easiest way to lose weight (2 cups water equals 2 pounds). Most common practices to lose water weight include fluid restriction, exercising in hot and humid conditions, and the use of saunas, "sauna suits," or diuretics. Risks of dehydration include irritability, dizziness, fatigue, weakness, organ failure, and death.

c. Abuse of diuretics and/or laxatives. Used to reduce further the body of excess "weight." This method combines all the risks of dehydration and starvation by depriving the body of fluids and nutrition.

d. Vomiting and/or purging. May lead to dehydration and can be self-induced or with emetics (laxatives) that stimulate the response. This method combines all the risk of dehydration and starvation by depriving the body of fluids and nutrition.

e. Use of diet or weight loss pills (appetite suppressants, metabolism boosters, fat burners).

(1) These weight loss aids may contain chemicals that act like drugs. Many of these supplements can be lethal, especially when taken before heightened physical activity. Others may result in serious side effects like liver damage, kidney problems, heart failure, stroke, or extreme dehydration. Supplements may have negative interactions with medications, other supplements, or existing medical conditions. The supplement may not have been proven to have any effect on weight loss.

(2) Unlike pharmaceutical products, manufacturers do not need to register dietary supplements with the Food and Drug Administration (FDA) or get FDA approval before producing or selling their products. FDA cannot take action unless problems are reported after the supplement is marketed.

C–5. Unsafe diets

Be suspicious of diets that—

a. Promise rapid weight loss.

b. Allow unlimited quantities of only certain foods and/or are overly strict.

c. Encourage unsafe practices such as fasting, use of diuretics and/or laxatives, or colon cleansing.

d. Promote special dietary supplements of "diet" pills.

C–6. Eating disorders

An eating disorder is an illness that causes serious disturbances to a person's food intake, such as eating extremely small amounts of food or severely overeating. Eating disorders affect both men and women, and result from a variety of emotional, physical, and social issues such as depression, anxiety disorders, or substance abuse. Although eating disorders may begin with a preoccupation with food and weight, they are more than just about food. Leaders who suspect a Soldier of suffering from an eating disorder should submit a referral for medical evaluation.

a. Anorexia nervosa. A serious potentially life-threatening eating disorder characterized by self-starvation and excessive weight loss. Individuals with anorexia nervosa see themselves as overweight even though they are clearly underweight. Eating, food, and weight control become obsessions.

b. Bulimia nervosa. Characterized by a cycle of bingeing and compensatory behaviors such as self-induced vomiting designed to undo or compensate for the effects of binge eating. Bulimia nervosa is a serious, potentially life-threatening eating disorder.

c. Binge eating. Occurs when a person loses control over his or her eating. Unlike bulimia nervosa, it is not followed by purging, excessive exercise, or fasting.

d. Eating disorders not otherwise specified. Eating disorders that include a combination of signs and symptoms but do not meet the full criteria for an eating disorder.

C–7. Resources

a. USAPHC TG 358. The Army Weight Management Guide at http://phc.amedd.army.mil/PHC%20Resource%20Library/USAPHC_TG_358_Army_Weight_Management_Guide.pdf provides a list of current nutrition and weight management resources.

b. De-mything diets. Diet books routinely top the bestseller lists and new fad diets frequently surface. The following Web sites sort out the myths to increase understanding of which diets are reasonable and which should be avoided:

(1) Academy of Nutrition and Dietetics at http://www.eatright.org/dietreviews.

(2) Weight Control Information Network at http://win.niddk.nih.gov/publications/myths.htm. View Web page "*Weight Loss and Nutrition Myths-How Much do you Know?*"

c. Weight loss programs. Weight Control Information Network at http://www.win.niddk.nih.gov/publications/choosing.htm. View Web page "*Choosing a Safe and Successful Weight Loss Program.*"

Appendix D
Internal Control Evaluation

D–1. Function
The function covered by this evaluation is the ABCP.

D–2. Purpose
The purpose of this evaluation is to assist the commanders, supervisors, and health care personnel in evaluating the key internal controls listed. It is intended as a guide and does not cover all controls.

D–3. Instructions
Answers must be based on the actual testing of key internal controls (for example, document analysis, direct observation, sampling, simulation, or other). Answers that indicate deficiencies must be explained and the corrective action identified in supporting documentation. These internal controls must be evaluated at least once every 2 years or whenever the internal control administrator changes. Certification that the evaluation has been conducted must be accomplished on DA Form 11–2 (Internal Control Evaluation Certification).

D–4. Test questions
a. Is there a master fitness trainer or has someone been designated as the unit fitness training NCO?

b. Has a height/weight and/or body fat assessment been performed and documented within the last 6 months for each Soldier in the unit not enrolled in the ABCP?

c. Did the commander enroll all eligible Soldiers exceeding body fat standards into the ABCP through notification counseling within 2 working days from initiation of the DA Form 268 for AC and RC Soldiers on active duty (the next MUTA for RC Soldiers not on active duty)?

d. Is there a completed unit ABCP file for Soldiers enrolled in the ABCP program?

e. Is there a DA Form 268 completed on Soldiers within 3 working days of being found noncompliant with body fat standards?

f. Is there a completed Soldier Action Plan on file within 14 days of the notification counseling?

g. Is nutrition counseling completed within 30 days after enrollment in the ABCP for AC and RC Soldiers on active duty?

h. Does monthly body fat assessment documentation exist for all Soldiers enrolled in the ABCP?

i. Are the Soldiers who perform the circumference-based tape method to determine Soldier body fat composition trained and competent to perform the measurements?

j. Is there a plan and/or policy established and maintained to describe how key internal controls will be evaluated over a 2-year period?

D–5. Supersession
Not applicable.

D–6. Comments
Help to make this a better tool for evaluating internal controls. Submit comments to Deputy Chief of Staff, G–1 (DAPE–HR), 300 Army Pentagon, Washington, DC 20310–0300.

Glossary

Section I
Abbreviations

ABCP
Army Body Composition Program

AC
Active Component

ACOM
Army command

ADT
active duty for training

AGR
Active Guard Reserve

APFT
Army Physical Fitness Test

AR
Army regulation

ARNG
Army National Guard

ASCC
Army service component command

CG
commanding general

DA
Department of the Army

DCS
Deputy Chief of Staff

DRU
direct reporting unit

FDA
Food and Drug Administration

FM
field manual

IRR
Individual Ready Reserve

MODS
Medical Operational Data System

MTF
medical treatment facility

MUTA
multiple unit training assembly

NCO
noncommissioned officer

NGR
National Guard Regulation

RC
Reserve Component

TG
technical guide

USAPHC
U.S. Army Public Health Command

USAR
U.S. Army Reserve

Section II
Terms

Body composition
Consists of two major elements of the human body: lean body-mass (which includes muscle, bone, and essential organ tissue) and body fat. Body fat is expressed as a percentage of total body weight that is fat. For example, an individual who weighs 200 pounds and has 18 percent body fat has 36 pounds of fat. Women generally have a higher percentage of body fat than men because of genetic and hormonal differences; thus, body fat standards differ among men and women by age groups.

Health care personnel
Trained physicians (military or civilian employees or contract personnel), physician's assistants, registered nurses, dietitians, and physical and/or occupational therapists under supervision of the unit surgeon or the commander of the MTF. For the purpose of this regulation, this term includes personnel of U.S. forces and host nations.

Exceed body fat standards
When a Soldier's percent body fat exceeds the standard specified in paragraph 3–2. Soldiers that exceed body fat standards are considered not in compliance with Army body fat standards.

Satisfactory progress
As described in paragraph 3-9*b*, progressing at a reasonable pace toward meeting the body fat standard. A monthly loss of 3 to 8 pounds or 1 percent body fat is required for satisfactory progress.

Section III
Special Abbreviations and Terms

Flag
suspension of favorable personnel action

Semi-structured Barriers Interview. Thoroughly evaluating possible barriers to study completion with potential participants prior to enrollment minimizes attrition. Additionally and when possible, a staged screening process that requires participants to complete two or more screening visits over a few weeks helps eliminate people who are less reliable and/or whose schedules are not conducive to study completion. Barriers interviews and multiple screening visits have worked well in other studies at Pennington Biomedical Research Center, including intensive and long inpatient studies.

During the current project, a Semi-structured Barriers Interview will be administered to potential participants to identify impediments to study completion and to build a consensus with the participant that, if they enroll, they will be committed to finishing the study and recognize that they are accountable to the study team and also that the study team is accountable to the participant. The interview provides a non-judgmental framework to work with the potential participant to determine if the study is consistent with their lifestyle/time demands and if adhering to and completing the study is feasible. Additionally, motivational factors for enrolling in the study are identified and discussed, including intrinsic motivations (e.g., helping others via research participation) and extrinsic motivations (e.g., monetary compensation).

The Barriers Interview will be administered by a staff member of Corby Martin, Ph.D., a licensed Clinical Psychologist, and each potential participant's case will be staffed with Kishore Gadde, MD and/or Dr. Martin prior to recommending inclusion or exclusion. The interview will be adapted for the specific needs of the present study and evaluate the following logistical barriers, as well as mental health/behavioral factors that could impede study completion. As noted below, the interview includes a psychological screen to identify the exclusionary criteria suggested by Dr. Gadde.

<u>Logistical Barriers:</u>

1. Planned vacation or work commitments that will result in being out of town during the time of enrollment.
2. Family obligations (e.g., care for a dependent) that inhibit study completion.
3. Pending employment with an unknown schedule or availability of time off.
4. Lack of transportation to and from the Center.

<u>Psychological and Behavioral Barriers:</u>

1. An inconsistent work history suggesting difficulty completing commitments.
2. Previous incarceration, particularly for a violent crime.
3. History of hospitalization for a mental health problem, alcohol or drug problem, violent behavior, or suicidal ideation (exclude if present).
4. Previous treatment for a mental health problem (Counseling is okay. If given medications, ask when, what and how long, and discuss with study physician. Cannot be taking any psychiatric medications within a month of screening.)
5. Belief that someone was plotting against you, or people were spying on you.
6. Feel as if someone implanted thoughts in your mind.
7. Heard voices other people couldn't hear.
8. Had visions or seen things others could not see.

9. Periods of being unusually 'up' or 'high' or 'hyper' or so full of energy that you got into trouble, or other people thought you were abnormally energetic.
10. History of feeling depressed, down in the dumps, or blue, or lost interest in most activities that you normally enjoy, most of the day, nearly every day, for 2 weeks or more?
    a. If yes, do you feeling like this now, or have felt like this very recently?
11. Ever had thoughts that life is not worth living or wish you were dead or better off dead?
12. Ever planned to harm yourself or attempted suicide, or harm someone else? (If yes in the past, exclude. If having current thoughts, discuss with study physician).
13. Frequent anxiety attacks, or extreme fear or discomfort being in closed places like staying inside an MRI machine for an hour. (Past history is okay. If current, discuss with study physician.
14. In the past 3 months, any episodes of excessive eating or binging on a lot of food within a short time while feeing compelled to eat although not feeling hungry?
    a. History of vomiting after overeating / binging?
15. History of anorexia nervosa or significant restriction of energy intake or excessive exercise to rid the body of calories.

Pennington Biomedical Research Center

FOOD INTAKE DIARY FORM

Subject ID: _____

Day and Date of Record: _____

Completed in the 5 days before admit to PBRC

Time	Meal	Place	Food or Drink	Amount	Description
Specify A.M. or P.M.	**B**-Breakfast **L**-Lunch **D**-Dinner **S**-Snack	Home, school, restaurant (give name)	One item per line. Skip line between each meal or snack	Specify oz., cup, tsp, Tbsp, etc.	Be specific. Give details such as brand name, cooking method, etc. Attach recipes or food labels if applicable.
8am	*B*	*Home*	*Cornflakes*	*¾ cup*	*Kellogg's brand*
			Skim milk	*½ cup*	*Kleinpeter's*
			Toast	*1 slice*	*Cracked wheat, Albertson's brand*
			Margarine	*1 t*	*Country crock*
			Coffee	*8 oz*	*Community brewed decaf*
			Creamer	*1 t*	*Winn Dixie brand, powdered, light*
			sugar	*1 t*	*granulated*
12pm	*L*	*Work:*	*Sandwich:*		
		Cafeteria	*Turkey breast*	*2 slices*	*4"x3"x1/4"*
			Mayonnaise	*1 pkt*	*Regular, Kraft*
			Hoagie bun	*1 (large)*	*6" long, submarine type*
			Diet Coke	*12 oz*	*Caffeine free, no ice, came from vending machine*
			Orange, fresh	*1*	*3" diameter*
6pm	*D*	*Home*	*Chicken breast*	*1 medium*	*Battered, fried, with skin, ate the skin*
			Rice	*¾ cup*	*Plain white rice, cooked with salt added*
			Green beans	*½ cup*	*Libby's brand canned, cooked with bacon added*
			Iced tea	*10 oz*	*Luzianne brand, brewed, ½ of glass filled with ice, no sugar*
			White cake, Pillsbury mix	*1 piece*	*2"x3"x1" made from box with store brand (Shur Fine) canned white frosting*
8:30pm	*S*	*Home*	*Popcorn*	*Bag*	*3oz bag of Pop Secret, reduced fat, microwaved*
			Beer, light	*24 oz*	*2 cans of Budweiser light beer*
			Peanuts	*Handful*	*Measured approximately ½ cup, salted, shelled*

Pennington Biomedical IRB FWA 00006218
Approved 12/16/15

Pennington Biomedical Research Center

STUDY NAME

FOOD INTAKE DIARY FORM

Subject ID: _____

Day and Date of Record: _____

Completed in the 5 days before admit to PBRC

Time	**Meal**	**Place**	**Food or Drink**	**Amount**	**Description**
Specify A.M. or P.M.	**B**-Breakfast **L**-Lunch **D**-Dinner **S**-Snack	Home, school, restaurant (give name)	One item per line. Skip line between each meal or snack	Specify oz., cup, tsp, Tbsp, etc.	Be specific. Give details such as brand name, cooking method, etc. Attach recipes or food labels if applicable.

Appendix E. Biological sample time point collection.

Blood samples

Analyte[1]	SV	0	7	14	28	42	43	56	EOS	EOS+1	PRN
PBRC											
Total testosterone	X	X		X	X	X		X	X		X
Free testosterone	X	X		X	X	X		X	X		X
LH		X		X	X	X		X	X		
FSH		X		X	X	X		X	X		
SHBG		X		X	X	X		X	X		
IGF-1		X		X	X	X		X	X		
Estradiol		X		X	X	X		X	X		
Insulin[2]		X	X	X	X	X	X	X	X	X	
Cortisol		X		X	X	X		X	X		
PSA	X			X		X					
CBC	X	X		X	X	X		X	X		
Chem 26	X										
Chem 15		X		X		X		X	X		
Amino acids		X		X		X		X	X		
LBP		X		X		X		X	X		
Leptin[2]			X				X			X	
Glucose[3,4]			X				X			X	
Acyl ghrelin[3,4]			X				X			X	
Des-acyl ghrelin[3,4]			X				X			X	
USARIEM[5]											
FSR				X	X	X					
Body water				X	X	X					
Myosyntax[6]											
Plasma sample				X		X					
PBRC (archives)											
Lp(a)		X		X		X		X	X		
Hepcidin		X		X		X		X	X		
Interleukin-6		X		X		X		X	X		
STFR		X		X		X		X	X		
Ferritin		X		X		X		X	X		
Oxytocin		X		X	X	X		X	X		
DHEA-S		X		X	X	X		X	X		
Prolactin		X		X	X	X		X	X		
Vasopressin		X		X	X	X		X	X		
Melatonin		X		X	X	X		X	X		
PYY[3,4]			X				X			X	
GLP-1[3,4]			X				X			X	
Archive[3,4,7]	X	X	X	X	X	X	X	X	X	X	
Metabolon[8] (archives)											
Metabolomics		X		X		X		X	X		

Muscle biopsy samples

	14	28	42
USARIEM[5]			
Muscle tissue	X	X	X
Myosyntax[6]			
Muscle tissue	X		X

Saliva samples

	3	7	11	15	19	23	27	31	35	39
USARIEM[5]										
Saliva	X	X	X	X	X	X	X	X	X	X

Urine samples

PBRC	**SV**		**11**	**39**			**53**			**EOS**		
Urinalysis	X											
Drug screen	X											
Nitrogen			X	X			X			X		
Creatinine			X	X			X			X		
Mannitol			X	X						X		
Sucralose			X	X						X		
USARIEM[5]	**11**	**13**	**14**	**39**	**41**	**42**	**53**	**55**	**56**	**EOS**	**EOS+2**	**EOS+3**
Creatine	X	X	X	X	X	X	X	X	X	X	X	X

Fecal samples

	11	25	39	EOS
Fecal samples[9]	X	X	X	X

[1]Analyte abbreviations: Luteinizing hormone (LH), follicle-stimulating hormone (FSH), sex hormone binding globulin (SHBG), insulin-like growth factor-1 (IGF-1), dehydroepiandrosterone-sulfate (DHEA-S), complete blood count with differential (CBC w/diff), lipoprotein little a [Lp(a)], soluble transferrin receptor (STFR), lipopolysaccharide-binding protein (LBP), glucagon-like peptide-1 (GLP-1); peptide-YY (PYY).
[2]Measured on days 7, 43, and EOS+1 at minute -15 relative to consumption of a standard meal at 0 min.
[3]Measured on days 7 and EOS+1 at minutes -15, 30, 60, 120, and 180 relative to consumption of a standard meal at 0 min.
[4]Measured on day 43 at minutes -15, 30, 60, 120, 180, 215, 245, 305, and 365 relative to consumption of a standard meal at 0 min.
[5]De-identified samples will be shipped to Kinemed Inc. (Emeryville, CA) for assessment of body water enrichment.
[6]De-identified samples will be shipped to MyoSyntax (Boston, MA).
[7]Archived blood (serum and plasma) samples will be used for potential analyses of tertiary study related variables, including biomarkers of bone turnover, bone adaptations to stress, and thyroid function.
[8]De-identified samples will be shipped to Metabolon Inc. (Durham, NC) for global metabolomics assessments.
[9]Samples will be collected on the indicated day or within 72 hours thereafter, and will be initially processed at PBRC and stored frozen until analysis. De-identified samples will be shipped to Metabolon Inc. (Durham, NC) for global metabolomics assessments. De-identified samples will be shipped to the US Army Natick Soldier Research Development and Engineering Center for short-chain fatty acid concentration analysis. De-identified samples will be shipped to the Broad Institute (Cambridge, MA) for 16s gene sequencing. Remaining de-identified samples will be stored indefinitely in the PBRC archives to assess biomarkers associated with the study outcomes.

Appendix F: Calisthenics Circuit

Movement	Example Exercise	Description (FM 7-22)	Weight
Lifting from the ground	Sumo squat	pg 300-301	50 lbs
Pulling/Climbing	Pull-ups	pg 306	N/A
Lifting overhead	Overhead Push Press	pg 310	2 x 30 lbs
Lunging	Forward lunge	pg 303	2 x 20 lbs
Pushing	Push-up	pg 234	N/A

XX-XX; Physiological and psychological effects of testosterone during severe energy deficit and recovery.
PI: Jennifer Rood, PhD.

APPENDIX H

PBRC 6-day Cycle Menu (1.6 g/kg; ~20% PRO, 50% CHO, 30% fat)					
Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
Grits Butter, salted Canadian Bacon Egg, whites Orange Juice Milk, nonfat	Omelet: Egg, whites Spinach Tomatoes Cheese, ched Butter, salted Bagel Cream cheese Jam, strawberry Milk, nonfat	Greek Yogurt, Nonfat Almonds Blueberries, raw Cereal, oats Egg, whites Butter, salted Grapefruit Juice	Special K Cereal Milk, nonfat Banana, raw Turkey Sausage Eggs, whole Orange Juice	English Muffin, wheat Butter, salted Egg, whole Canadian Bacon Cheese, Cheddar Milk, nonfat Apple Juice	Waffles Butter, salted Maple Syrup Turkey Sausage Egg, whites Fruit Salad Milk, nonfat
Turkey Pita Pocket: Deli Turkey Mayo, regular Cheese, swiss Lettuce, romaine Tomatoes, slice Pretzels	Hamburger: Beef, ground Bun Mayo, nonfat Mustard Lettuce, romaine Tomatoes, sliced Crackers, wheat Cheese, swiss Grapes	Tuna Salad Sandwich: Tuna Salad Mayo, light Bread, wheat Lettuce, romaine Tomatoes, raw Cheese, cheddar Fruit Salad Sun Chips, multigrain	Chicken Caesar Wrap: Lettuce, romaine Chicken, diced Cheese, parm Caesar Dressing Tortilla, flour Tomatoes, raw Apple, raw	Roast beef Sandwich: Roast beef Bread, wheat Cheese, mozz Tomatoes, raw Lettuce, romaine Mustard Carrots, raw Ranch Dressing Tomato Soup Milk, nonfat	Chicken Tacos with Rice & Beans: Tortillas Chicken Lime Juice Cheese, monterey Salsa Lettuce, romaine Tomatoes, raw Spanish Rice Black Bean Turtle Soup Tropical Fruit Bowl
Lemon Sage Chicken: Olive oil Long-Grain Rice Mixed Vegetables Dinner Roll, wheat Butter, salted Pears, canned Milk, nonfat	Chicken Pesto Pasta: Spaghetti Chicken Pesto Cheese, parm Broccoli Banana Pudding	Pork Chop Rosemary Garlic Potatoes Carrots, cooked Butter, salted Dinner roll, wheat	Mexican Casserole: Beef, ground Black Bean Turtle Soup Salsa Cheese, cheddar Sour Cream Tortilla Chips Pineapple	Chicken spaghetti: Spaghetti, wheat Chicken Spaghetti Sauce Cheese, parm Lettuce, romaine Tomato, raw Cucumber, raw Italian Dressing Wheat Dinner Roll Butter, salted	Citrus Cod: Fish, cod Lemon Juice Orange Peel Butter, salted Green Beans Baked Potato Sour Cream Butter, salted Shortbread Cookie
Trail Mix: Crackers, goldfish Pretzels, mini Raisins Candy, M&Ms Granola Bar	Cookies, choc chip Apple Potato Chips, baked	Nutrigrain bar Peaches, canned Animal Crackers	Chocolate Pudding Vanilla Wafer Cookies Pear	Graham Crackers Grapes Marshmallow Fluff	Bread, White Chocolate-Hazelnut Spread Banana, raw

Pennington Biomedical Research Center
PARTICIPANT'S DAILY CHECKLIST

Name:_____ Date:_____

Please answer all questions below and place an "X" in the appropriate column. Please fill in the additional information requested when necessary. Please return this form each day to your monitor.

1. ____ ____ Were there any study foods you did not eat/drink on this day?
Yes No Reasons include missing, spilled, or inedible food, illness or other.

Food/Drink	Amount	Reason

2. ____ ____ Did you eat any foods that were not provided by the Research
Yes No Kitchen on this day? If yes, please name the food (be very specific), the amount, and reason consumed.

Food	Amount	Reason

3. ____ ____ Did you consume any *coffee, tea, or soda* on this day? **Please include**
Yes No **what was provided to you by PBRC.**

Beverage	Amount	Caffeinated?
	_____ oz.	
	_____ oz.	
	_____ oz.	
	_____ oz.	
	_____ oz.	

4. ____ ____ Did you drink any alcohol on this day? If yes, please record.
Yes No

Kind of Alcohol	Amount
	_____ oz.
	_____ oz.

5. ____ ____ Did you take any new medications or have any problems?
Yes No *If yes, call study coordinator*

6. ____ ____ Is there anything you would like us to know in relation to your
Yes No participation in this study?

PENNINGTON BIOMEDICAL RESEARCH CENTER LSU

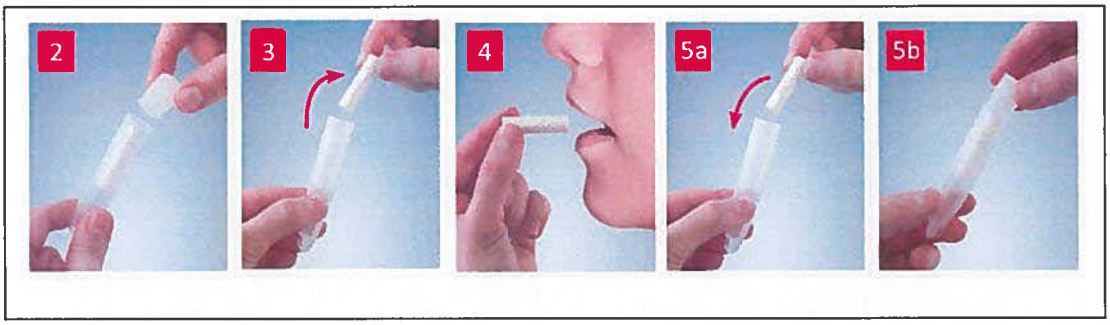
OPS

SALIVA COLLECTION INSTRUCTIONS

Please keep all unused salivettes (Sarstedt Cat # 51.1534) stored at room temperature. Do not store unused salivettes in the freezer as these will be difficult to saturate.

Collection Instructions:

1. If participant has had **any** food or drink within the past 30 minutes before producing this saliva sample, **do not continue**. Saliva must be collected at least 30 minutes after eating or drinking anything, including heavy water
2. Open the top lid of the plastic salivette vial by popping the lid off sideways.
3. Remove the white cotton swab from inside the vial and place it in participant's mouth.
4. Instruct participant to chew and suck on the cotton swab for 30-60 seconds, until the swab is saturated with saliva.
5. Place the wet swab back inside the salivette vial (a) and replace the cap, making sure it's securely on (b).



6. Do not allow the participant to spit into the plastic salivette vial at any time.
7. Record the collection date and time and participant ID on the salivette vial label.

Subject ___ ___ ___ - ___ ___

Date of Day 0:_____

PENNINGTON BIOMEDICAL RESEARCH CENTER LSU

OPS

Saliva Collection Journal

	Please fill in the date & time of each saliva collection.	Please note any deviation from instructions, such as missed collections, in the provided space below.
Day 3	**NO EATING OR DRINKING 30 MINS BEFORE** Date of collection: ____/_____/_____ Time of collection ___ : ___ am/pm (circle)	
Day 7	**NO EATING OR DRINKING 30 MINS BEFORE** Date of collection: ____/_____/_____ Time of collection ___ : ___ am/pm (circle)	
Day 11	**NO EATING OR DRINKING 30 MINS BEFORE** Date of collection: ____/_____/_____ Time of collection ___ : ___ am/pm (circle)	
Day 15	**NO EATING OR DRINKING 30 MINS BEFORE** Date of collection: ____/_____/_____ Time of collection ___ : ___ am/pm (circle)	
Day 19	**NO EATING OR DRINKING 30 MINS BEFORE** Date of collection: ____/_____/_____ Time of collection ___ : ___ am/pm (circle)	
Day 23	**NO EATING OR DRINKING 30 MINS BEFORE** Date of collection: ____/_____/_____ Time of collection ___ : ___ am/pm (circle)	
Day 27	**NO EATING OR DRINKING 30 MINS BEFORE** Date of collection: ____/_____/_____ Time of collection ___ : ___ am/pm (circle)	

Subject ___ ___ ___ - ___ ____

Date of Day 0:_____

OPS

	Please fill in the date & time of each saliva collection.	Please note any deviation from instructions, such as missed collections, in the provided space below.
Day 31	**NO EATING OR DRINKING 30 MINS BEFORE** Date of collection: ____/_____/_____ Time of collection ___ : ___ am/pm (circle)	
Day 35	**NO EATING OR DRINKING 30 MINS BEFORE** Date of collection: ____/_____/_____ Time of collection ___ : ___ am/pm (circle)	
Day 39	**NO EATING OR DRINKING 30 MINS BEFORE** Date of collection: ____/_____/_____ Time of collection ___ : ___ am/pm (circle)	

Subject ___ ___ ___ - ___ ___

PENNINGTON BIOMEDICAL RESEARCH CENTER LSU

OPS: Day 0 – Day 7

Heavy Water Intake Journal

Each bottle contains 50ml of heavy water.

Drink a total of 3 bottles of heavy water per day. Each bottle should be consumed at least 3 hours apart. The first bottle each day will be consumed before your breakfast meal at Pennington Biomedical Research Center. You will be given 2 additional bottles each day to consume with your lunch and dinner meals.

Keep and return all bottles (both used and unused) to study coordinator at each study visit. Please contact Wayne White at (225) 763-2690 if you have any questions/concerns.

Please fill in date for each day	Please fill in time of each heavy water dose consumption and check if entire bottle is consumed.	Please note any deviation from instructions, such as missed heavy water doses in the provided space below.
Day 0 ***Date:*** ***/ /*** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 0)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 0)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 3 (Day 0)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 1 ***Date:*** ***/ /*** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 1)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 1)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 3 (Day 1)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	

Subject ___ ___ ___ - ___ ___

PENNINGTON BIOMEDICAL RESEARCH CENTER
LSU

OPS: Day 0 – Day 7

Day	Dose	Comments
Day 2 ***Date:*** / / ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 2)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 2)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 3 (Day 2)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 3 ***Date:*** / / ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 3)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 3)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 3 (Day 3)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 4 ***Date:*** / / ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 4)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 4)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 3 (Day 4)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	

Subject ___ ___ ___ - ___ ___

PENNINGTON BIOMEDICAL RESEARCH CENTER LSU

OPS: Day 0 – Day 7

Day	Dose	Comments
Day 5 ***Date:*** ***/ /*** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 5)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 5)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 3 (Day 5)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 6 ***Date:*** ***/ /*** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 6)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 6)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 3 (Day 6)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 7 ***Date:*** ***/ /*** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 7)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 7)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 3 (Day 7)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	

Subject ___ ___ ___ - ___ ___

OPS: Day 8 – Day 42

Heavy Water Intake Journal

Each bottle contains 50ml of heavy water.

Drink a total of 2 bottles of heavy water per day. Each bottle should be consumed at least 3 hours apart.

The first bottle each day will be consumed before your breakfast meal at Pennington Biomedical Research Center. You will be given an additional bottle each day to consume with your dinner meal.

Keep and return all bottles (both used and unused) to study coordinator at each study visit. Please contact Wayne White at (225) 763-2690 if you have any questions/concerns.

Please fill in date for each day	Please fill in time of each heavy water dose consumption and check if entire bottle consumed.	Please note any deviation from instructions, such as missed heavy water doses, in the provided space below.
Day 8 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 8)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 8)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 9 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 9)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 9)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 10 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 10)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 10)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	

Subject ___ ___ ___ - ___ ___

OPS: Day 8 – Day 42

Day	Dose	Comments
Day 11 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 11)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 11)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 12 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 12)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 12)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 13 ***Date: / /*** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 13)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 13)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 14 ***Date: / /*** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 14)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 14)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 15 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 15)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 15)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	

Subject ___ ___ ___ - ___ ___

OPS: Day 8 – Day 42

Day	Dose	Comments
Day 16 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 16)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 16)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 17 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 17)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 17)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 18 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 18)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 18)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 19 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 19)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 19)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 20 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 20)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 20)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	

Subject ___ ___ ___ - ___ ___

OPS: Day 8 – Day 42

Day	Dose	Comments
Day 21 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 21)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 21)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 22 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 22)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 22)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 23 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 23)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 23)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 24 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 24)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 24)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 25 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 25)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 25)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	

Subject ___ ___ ___ - ___ ___

OPS: Day 8 – Day 42

Day	Dose	Comments
Day 26 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 26)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes ☐ No ☐ **Water Bottle 2 (Day 26)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes ☐ No ☐	
Day 27 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 27)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes ☐ No ☐ **Water Bottle 2 (Day 27)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes ☐ No ☐	
Day 28 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 28)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes ☐ No ☐ **Water Bottle 2 (Day 28)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes ☐ No ☐	
Day 29 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 29)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes ☐ No ☐ **Water Bottle 2 (Day 29)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes ☐ No ☐	
Day 30 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 30)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes ☐ No ☐ **Water Bottle 2 (Day 30)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes ☐ No ☐	

Subject ___ ___ ___ - ___ ___

OPS: Day 8 – Day 42

Day	Dose	Comments
Day 31 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 31)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 31)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 32 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 32)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 32)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 33 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 33)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 33)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 34 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 34)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 34)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 35 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 35)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 35)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	

Subject ___ ___ ___ - ___ ___

OPS: Day 8 – Day 42

Day	Dose	Comments
Day 36 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 36)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 36)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 37 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 37)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 37)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 38 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 38)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 38)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 39 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 39)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 39)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 40 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 40)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 40)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	

Subject __ __ __ - __ __

OPS: Day 8 – Day 42

Day	Dose	Comments
Day 41 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 41)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 41)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	
Day 42 **Date: / /** ***Take each bottle at least 3 hours apart***	**Water Bottle 1 (Day 42)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □ **Water Bottle 2 (Day 42)** Time of consumption ___ : ___ am/pm (circle) All consumed? Yes □ No □	

Pennington Biomedical IRB FWA 00006218
Approved 12/16/15

Place Barcode Label Here

Biodex Performance Data Form

DAY(Circle): 0 1 2 3 4 5

File Name: _____

Isometric Force Test:

(2 sets practice at ~50% and ~75% of maximal effort; 3 Set at maximal effort at 75°)

Reps	Peak Torque	Time to Peak Torque	10% Peak Torque Time	40% Peak Torque Time
1	_____Nm	_____msec	_____ msec	_____ msec
2	_____Nm	_____msec	_____ msec	_____ msec
3	_____Nm	_____msec	_____ msec	_____ msec

Isokinetic Strength Test:

(1 set of 6 reps practice at ~50-75% of maximal effort; 1 sets of 6 maximal reps/test; 60°/sec)

Set	Reps	Peak Torque	Angle at Peak Torque	Average Torque
1	6	_____Nm	_____°	_____Nm

Isokinetic Endurance Test:

(1 set of 5 reps practice at ~50-75% of maximal effort; 1 set of 20 maximal reps/test; 180°/sec)

Set	Reps	Peak Torque	Angle at Peak Torque	Average Torque	Total Work
1	20	_____Nm	_____°	_____Nm	_____W

Fatigue Index

Highest	Extension
1	
2	
3	
4	
5	

Lowest	Extension
1	
2	
3	
4	
5	

Data Entry (Initials/Date): _____ **Quality Control (Initials/Date):** _____

Pennington Biomedical IRB FWA 00006218
Approved 12/16/15

THE AGGRESSION QUESTIONNAIRE

Rate each of the following items in terms of how characteristic they are of you. Use the following scale:

1	2	3	4	5
Extremely uncharacteristic of me				Extremely characteristic of me

Aggression Factor I

_____ 1. Once in a while, I can't control the urge to strike another person.

_____ 2. Given enough provocation, I may hit another person.

_____ 3. If someone hits me, I hot back.

_____ 4. I get into fights a little more than the average person.

_____ 5. If I have to resort to violence to protect my rights, I will.

_____ 6. There are people who pushed me so far that we came to blows.

_____ 7. I can think of no good reason for ever hitting a person.*

_____ 8. I have threatened people I know.

_____ 9. I have become so mad that I have broken things.

_____ **Total** (*Reverse rating 1 = 5, 2 = 4, 3 = 3. 4 = 2, 5 = 1)

Aggression Factor II

_____ 1. I tell my friends openly when I disagree with them.

_____ 2. I often find myself disagreeing with people.

_____ 3. When people annoy me, I may tell them what I think of them.

_____ 4. I can't help getting into arguments when people disagree with me.

_____ 5. My friends say that I'm somewhat argumentative.

_____ **Total**

Rate each of the following items in terms of how characteristic they are of you. Use the following scale:

1	2	3	4	5
Extremely uncharacteristic of me				Extremely characteristic of me

Aggression Factor III

_____ **1**. I flare up quickly but get over it quickly.

_____ 2. When frustrated, I let my irritation show.

_____ 3. I sometimes feel like a powder keg ready to explode.

_____ 4. I am an even-tempered person.*

_____ 5. Some of my friends think I'm a hothead.

_____ 6. Sometimes I fly off the handle for no good reason.

_____ 7. I have trouble controlling my temper.

_____ **Total** (*Reverse rating 1 = 5, 2 = 4, 3 = 3. 4 = 2, 5 = 1)

Aggression Factor IV

_____ 1. I am sometimes eaten up with jealousy.

_____ 2. At times I feel I have gotten a raw deal out of life.

_____ 3. Other people always seem to get the breaks.

_____ 4. I wonder why sometimes I feel so bitter about things.

_____ 5. I know that "friends" talk about me behind my back.

_____ 6. I am suspicious of overly friendly strangers.

_____ 7. I sometimes feel that people are laughing at me behind my back.

_____ 8. When people are especially nice, I wonder what they want.

_____ **Total**

Buss, A. H. & Perry, M. P. (1992). The Aggression Questionnaire. Journal of Personality and Social Psychology, 63, 452-459.

TALLY SHEET

Gender:

_____Male _____Female

TOTAL SCORE FOR EACH FACTOR				
I	**II**	**III**	**IV**	**TOTAL SCORE**

Buss, A. H. & Perry, M. P. (1992). The Aggression Questionnaire. Journal of Personality and Social Psychology, 63, 452-459.

ABSTRACT

A new questionnaire on aggression was constructed. Replicated factor analyses yielded 4 scales: {Physical Aggression, Verbal Aggression, Anger and Hostility. Correlational analysis revealed that anger is the bridge between both physical and verbal aggression and hostility. The scales showed internal consistency and stability over time.

Men scored slightly higher on Verbal Aggression and Hostility and much higher on Physical Aggression. There was no sex difference for Anger (Table).

The various scales correlated differently with various personality traits. Scale scores correlated with peer nominations of the various kinds of aggression. These findings suggest the need to assess not only overall aggression but also its individual components.

TABLE

MEANS FOR THE AGGRESSION QUESTIONNAIRE

	Males (n= 612)	**Males**	**Females** (n = 641)	**Females**
Physical Aggression (I)	**24.3**		**17.9**	
Verbal Aggression (II)	**15.2**		**13.5**	
Anger (III)	**17.0**		**16.7**	
Hostility (IV)	**21.3**		**20.2**	
TOTAL	**77.8**		**68.2**	

Physiological and psychological effects of testosterone during severe energy deficit and recovery: a randomized, placebo controlled trial. PI: Jennifer Rood, PhD

Instructions: Each question is accompanied by a straight line. This line may be thought of as a continuum, with possible responses ranging from "not at all" at the left end through "extremely" at the right end. Please place a small vertical mark crossing this line wherever you think is appropriate based on how you feel at this moment. You may place your mark anywhere along this line. If you are indifferent or want your response to be "neither", then you can place your mark right in the middle.

How hungry are you right now?

Not at all hungry _____ Extremely hungry

How full are you right now?

Not at all full _____ Extremely full

How much do you think you could eat right now?

Nothing at all _____ A very large amount

How strong is your desire to eat?

Very weak _____ Very strong

VOLUNTEER ID: _____

DATE: _____

STUDY DAY: _____

TIME POINT: _____

Pennington Biomedical IRB FWA 00006218
Approved 4/19/16

PENNINGTON BIOMEDICAL RESEARCH CENTER LSU

OPS Study

Fecal Collection Instructions

Each collection kit is packaged in a drawstring bag and includes a plastic collection container for the toilet bowl, a 30ml graduated stool sample transport tube, a wooden tongue depressor to transfer stool into the tube, and a biohazard zip-close bag with an absorbent sheet for specimen transport. **Since the sample must be processed within 12 hours of collection, you are encouraged to collect stool during a study visit at Pennington.**

To collect the stool specimen:

1. Remove the contents of the collection kit from the drawstring bag.
2. Lift the toilet seat.
3. Place the plastic collection container over the toilet bowl.
4. Lower the toilet seat.
5. Collect the stool in the plastic collection container.
   - Do not to contaminate the stool with urine, toilet water, or toilet paper.
   - Do not pass the specimen directly into the transport tube.
6. Use the wooden tongue depressor provided to transfer approximately 5ml of stool to the 30ml graduated stool sample tube provided.
7. Secure the screw cap on the 30ml graduated stool sample tube taking care not to cross thread the cap.
8. Document the collection date and collection time on the label directly on the 30ml graduated stool sample tube.
9. Seal the stool sample tube in the zip-close specimen transport bag containing a small absorbent sheet.
10. Excess stool in the plastic collection container should be discarded in the toilet.
11. Place the used collection container and the wooden tongue depressor in the drawstring bag, tie shut, and dispose in the trash.
12. Wash your hands thoroughly after collecting the specimen.

If you are unable to provide a stool sample while you are at PBRC, you will be instructed to collect a sample at home within 72 hours of the study visit. In order to maintain the quality of the stool sample, **it must remain chilled from the time of collection until it is received at Pennington Biomedical, and it must be delivered to PBRC within 12 hours of collection.** If the time between collection and processing exceeds 12 hours, the sample cannot be used for analysis, and you will be asked to collect another sample.

Notify your study coordinator, Wayne White at (225) 763-2690 if you have any questions or concerns.

Appendix L: Detailed Description of the Clinical Research Facilities at Pennington Biomedical Research Center

The Pennington Biomedical Research Center (PBRC) is a model for clinical and translational research, since it houses basic, clinical and population research programs in one facility. The description here shall focus on the Clinical Research Unit.

The outpatient-based clinical research of PBRC is located in Clinical Research Building No. 1. Clinical Research Building No. 1 is a four-story, 90,000 GSF building that connects on the 2nd floor to Clinical Research Building No. 2. The facilities at PBRC are dedicated solely to the clinical research trials for the PBRC. The unit is designed for easy access and convenience of research volunteers. The PBRC's central location in Baton Rouge, covered drive-up entry and spacious free parking are assets encouraging research subject participation. Accommodations for on-site dining and convenient take-out food service and a food delivery service facilitate feeding studies. The activity of the Clinic over the years 1992-2014 is summarized below in Table A.

The ground floor of Clinical Research Building No. 1 includes 18 general examination rooms, 2 EKG procedure rooms, one room equipped for determination of height, weight and anthropometrics, 3 private interview rooms and a secured pharmacy storage room. Also on the ground floor is the phlebotomy area. This area includes 6 phlebotomy chairs with 4 connecting specimen collection facilities. The medical records library houses 3 offices and a secured storage space for medical records. The clinical unit also contains administrative office space, reception and waiting areas, 6 recruiting offices and 3 offices for physician personnel.

An Imaging Core Laboratory with DXA instrumentation, an ECHO MRI and Ultrasound testing rooms is also located on the ground floor. In the Imaging Laboratory there is also office space for 2 technicians. In addition, an exercise lab for conducting ECG monitored maximal exercise testing (i.e. VO2 Max) is found on the ground floor. The exercise lab has men's and women's locker rooms and facilities.

The second floor of Clinical Research Building No. 1 has a psychology behavioral area that accommodates 5 eating monitors and offices for the personnel. Since the second floor connects directly to first and second floors of Clinical Research Building No. 2 at the Research Kitchen area, all participants eating and undergoing monitored eating are conducted adjacent to the Research Kitchen. There is a participant dining area located on the 2nd floor which accommodates approximately 30 people.

The Clinical Research Building No. 1 also contains the following:

- One floor for additional support personnel for expanded clinical activities
- Housing for the home office of the Louisiana Clinical and Translational Science Center (LACATS)
- Housing for up to forty (40) Study Coordinators
- Office space for five (5) physicians and fifteen (15) faculty members and their support staff

The second floor of Clinical Research Building No. 2 contains the following:

- Research Kitchen (described in more detail below)
- Four indirect room-size calorimeters for the Metabolic Chamber Core (described in more detail below)
- Indirect calorimetry suite (seven stations total)
- Inpatient dining area
- Ten inpatient rooms (each with two beds/room)
- Nurses' station
- Satellite pharmacy
- Specimen processing room
- Sunroom for participant utilization
- Three rooms for Euglycemic and hyperglycemic clamp procedures
- IV Procedure room (seven chairs) for FSIGTT, oral glucose tolerance testing and meal tests
- Biopsy room for skeletal muscle and adipose biopsy

A recent renovation of Clinical Research Building No. 2 included conversion of the facilities on the first floor to a pediatric research clinic and updating the inpatient facilities on the second floor, including a procedure suite, metabolic cart suite, three additional inpatient rooms (two beds each) and two additional metabolic chambers. The renovation for the outpatient pediatric research unit included exam and treatment areas, procedure rooms, a metabolic cart room, meeting room and demo kitchen, indoor play and observation area, outdoor play area, exercise room, medical records storage for pediatric charts only, lab and phlebotomy area, administrative and other office support. The total renovation included 14,150 square feet of pediatric research area.

Additional expansion of the PBRC facilities included the construction of a new 30,000 SF Imaging Center that connects to the Clinical Research Building No. 1. This new Imaging Center was completed in April 2012 and accommodates Magnetic Resonance Imaging, x-ray and other imaging equipment as well as faculty offices and support space.

The following units act as Clinical Research Core Services at Pennington Biomedical Research Center:

1. Clinical Trials Unit
2. Recruiting Core
3. Biostatistics and Data Management Core
4. Clinical Chemistry Core
5. Mass Spectrometry Laboratory
6. Dietary Assessment Core
7. Ingestive Behavior Laboratory
8. Psychological Assessment Laboratory
9. Metabolic Assessment Core
10. Research Kitchen Core
11. Imaging Core

12. Exercise Testing Core

Clinical Trials Unit
The Clinical Trials Unit is directed by Robert Leonhard, MBA. The unit's Chief Medical Officer is Frank Greenway, M.D. and the Medical Director is Kishore Gadde, M.D. The unit is staffed with an additional medical doctor, physician's assistant, nurse practitioner, registered nurses, licensed practical nurses, registered dietitians and study coordinators. The unit is responsible for the oversight and coordination of all clinical trials performed at the center for both adult and pediatric studies.

The outpatient clinic of the Clinical Trials Unit is open Monday through Friday from 7:00 am until 4:30 pm. The Outpatient Unit provides the following services but is not limited to: screening of potential study participants, completion of protocol specific clinic visits, regulatory oversight for all studies, study specific coordinators and back-up coordinators, dispensing of study medications, completion of case report forms and quality assurance of source documentation. The unit is comprised of the general examination rooms, interview rooms, phlebotomy area and pharmacy previously mentioned.

The inpatient clinic of the Clinical Trials Unit is open seven days a week including holidays, with the exception of the Christmas break. The inpatient clinic provides the following services but is not limited to: euglycemic clamps, oral glucose tolerance tests, lumbar punctures, frequently sampled insulin glucose testing, meal tolerance testing, feeding studies, pharmacokinetic testing (including phase I-IV medication trials), muscle and fat biopsies, and overnight stays. The unit is able to perform extended inpatient testing as necessary. All licensed medical personnel are ACLS and BLS certified. Renovation of the unit was completed in 2013. The unit has ten inpatient rooms (twenty subject capacity), three euglycemic clamp rooms, a large procedure room, biopsy room, satellite pharmacy, blood specimen processing room, and functional nurses' station equipped with a telemetry system. The procedure statistics by year are listed above.

Recruiting Core
Recruitment services for clinical trials and other human research studies conducted at PBRC are coordinated by the Recruitment Core and directed by Brandi Bourgeois. The Recruitment Core manages all marketing activities for human research studies at PBRC, such as the design and placement of advertisement, and screens all incoming calls to determine study eligibility. Incoming calls are directed to a call center that is operated by 3 full time recruiters and is equipped with a Uniform Call Distributor (UCD) system. A UCD system expands the capability of a traditional phone system and allows multiple individuals to call simultaneously and be directed to the next available recruiter. The core utilizes an electronic message tracking application that tracks the outgoing phone call activity and a "smart" electronic phone screen system that screens potential participants upon initial phone contact and seamlessly matches them to alternative studies when deemed ineligible for the original study that the participant called. In 2012 the core launched a new web-screener for participants to be able to go on-line, choose a study that are interested in and complete a preliminary screening. The system is able

to tell the participant upon completion whether they are eligible to that point in the screening process and if they are ineligible the screener will alert them to other studies that they be eligible for and at that point could continue to screen for those studies. If the participant is eligible they are then contacted by a live recruiter to complete the screening process and schedule their first screening appointment. In 2014 an average of 829 web screens were completed each month with just under 10,000 total web-screeners for the year.

All marketing activities are coordinated by the Marketing and Outreach Coordinator, Denise Bourgeois Traditional advertisement mediums that are utilized include newspaper, television, and direct mailing, while more novel methods have been employed including online advertisement and mass email campaigns. A listserv was developed to manage email campaigns targeting the 20,000+ subscribers who have opted to be notified of new studies via email. In addition to online marketing, the core utilizes social media outlets such as Facebook, Twitter, Instagram, YouTube etc. to engage community members in participation in the research studies. The core also has access to demographic information for approximately 35,000 subjects who can be targeted for future studies.

Biostatistics and Data Management and Analysis Capabilities
The Pennington Center Biostatistics and Data Management Core is headed by William Johnson, Ph.D. This core resides in the Population Science research program at PBRC, which is headed by Peter Katzmarzyk, Ph.D. In addition to Johnson and Katzmarzyk, there are two Ph.D. and five master level biostatisticians. Together, this team serves the research design and analysis needs for 85 faculty members at PBRC. The Core is housed in the Clinical Research Building and there are spacious offices for faculty and adjacent cubicles for support personnel. The Core is equipped with Pentium computers and the E-mail and data transfer needs are supported by the PBRC Technology Services Group. The standard software used for statistical analysis is the most recent version of SAS, presently Version 9.4. Other software, such as spreadsheets and word processing packages, are used routinely. All computers used by statisticians are connected with the HP 990Cse color printer.
The Biostatistics and Data Management Core seeks collaborations that lead to a smooth transition from hypothesis formulation to efficient research study design and execution through quality-controlled data management, statistical analysis and summary presentations. Our overarching goal is to create electronic databases that accurately describe research outcomes and provide state-of-the-art statistical techniques for the objective interpretation of research findings that are captured in the observed data.

The data management team serves as a comprehensive clinical data coordinating facility. Their primary responsibility is the continuing development of a proprietary web-based portal to the clinical research database. The team interfaces with researchers to ensure the efficient and accurate transfer of data from observation to electronic files for storage and analysis; monitors the data processing throughout each study's duration; and provides investigators with study specific data sets via web-based desktop data

access. The team has developed custom applications for expedited creation of study specific data sets that may contain both PBRC data and Non-PBRC data. This development and data storage paradigm allows the team to work with both intramural and extramural researchers.

Guidelines for Good Clinical Practices as they relate to data handling have been documented and implemented in daily tasks. The group maintains current HIPAA Security Rule training and works closely with the Director of Intellectual Property, Legal and Regulatory Affairs. Programmers are provided with the latest software and hardware which allow them to perform their work efficiently.

Clinical Research Laboratory
The Clinical Research Laboratory is directed by Jennifer Rood, Ph.D., DABCC, FACB. The laboratory is accredited by the Centers for Medicare and Medicaid Services (CMS/CLIA) and the College of American Pathologists (CAP). The laboratory also participates in the lipid standardization program offered by the Centers for Disease Control. Good Clinical Practices guidelines are being followed in the laboratory. The Clinical Research Laboratory at Pennington Biomedical Research Center performs analyses for PBRC clinical trials, for basic researchers at the Center, for the US Army Institute of Environmental Medicine (USARIEM), and for other contracting clients. The Clinical Research Laboratory is staffed by licensed medical technologists, licensed phlebotomists, and research project assistants. The laboratory is subdivided into the following departments: phlebotomy, accessioning, chemistry, hematology, urinalysis, special chemistry and point-of-care testing.

The laboratory is well-equipped for performing routine and specialized tests on clinical subjects. The laboratory offers more than 350 different assays, and is also involved in developing new methodologies.

The following instrumentation and assays are examples:

- **Agilent Technologies HPLC 1100** : 3-methylhistidine, alanine, alpha carotene, alpha tocopherol, asymmetric dimethylarginine, arginine, asparagine, beta carotene, bromide, cryptoxanthin, glutamic acid, glutamine, glutathione, glycine, histidine, isoleucine, leucine, lutein, lycopene, lysine, methionine, phenylalanine, retinol, serine, threonine, tryptophan, tyrosine, valine, zeaxanthin.
- **Alco Scan**: breath alcohol (point of care testing)
- **Antek 9000**: nitrogen (urine, fecal, sweat, and saliva)
- **Siemens Clinitek 50**: urine microalbumin
- **Siemens Clinitek 500**: urine blood, pH, glucose, ketones, leukocyte esterase, nitrite, protein, specific gravity, urobilinogen, bilirubin
- **Beckman Coulter Immage 800**: apolipoprotein A1, apolipoprotein B, Lp(a), prealbumin, transferrin
- **Beckman Coulter Unicel DxH 800**: red blood cell count, hemoglobin, hematocrit, MCV, MCH, MCHC, RDW, platelet count, mean platelet volume, white blood cell count, basophils, eosinophils, lymphocytes, monocytes, neutrophils, reticulocytes, absolute granulocytes

- **Beckman Coulter DXC600**: albumin, alkaline phosphatase, ALT, amylase, AST, blood urea nitrogen, calcium, carbon dioxide, chloride, cholesterol, creatine kinase, creatinine, direct bilirubin, glucose, GGT, HDL cholesterol, hemoglobin A1C, iron, lactate dehydrogenase, LDL cholesterol, magnesium, osmolality, phosphorus, potassium, sodium, total bilirubin, total iron binding capacity, % iron saturation, total protein, triglycerides, urea, uric acid, angiotensin converting enzyme, BHBA, caffeine, FRAP, free fatty acids, fructosamine, glycerol, lactate, lipase, vitamin C, urine alcohol, TAS
- **Spectramax Plus 384**: carbonyls, UPDG
- **DCA 2000**: hemoglobin A1C (point of care testing)
- **Cholestech LDX**: glucose, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides (point of care testing)
- **ELISA – Bio Rad Plate Reader, BIOTEK 405 LS Plate Washer**: endothelin-1, IGF-1, IGF-2, IGF-BP1, IGF-BP3, bone alkaline phosphatase, cortisol (saliva), DHEA (saliva), progesterone (saliva), testosterone (saliva), PAI-1, ox40 ligand, CTX, tartrate resistant acid phosphatase, PTH related peptide, SCD40L, VCAM, E-selectin, ICAM, gastric inhibitory peptide, hepcidin, mouse insulin, soluble transferrin receptor, SAA, FGF-21, FGF-23, osteocalcin, free testosterone
- **Bio Rad HPLC**: epinephrine, norepinephrine
- **Siemens Immulite 2000**: ACTH, cotinine, C-peptide, C-reactive protein (high sensitivity), cortisol, deoxypyridinoline, DHEAs, estradiol, ferritin, folate, free T3, free T4, FSH, growth hormone, homocysteine, insulin, LH, myoglobin, progesterone, prolactin, PSA, PTH, SHBG, Troponin I, T uptake, T3, T4, TSH, vitamin B12, thyroglobulin, total testosterone
- **IL ACL 8000**: factor VII, fibrinogen, prothrombin time
- **Lifescan Ultra One Touch 2**: glucose (point of care testing)
- **Luminex Labmap 100 and 200**: apolipoprotein AII, apolipoprotein CIII, IL-1□, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, G-CSF, GM-CSF, IFN-□, MCP1, MIP-1□ TNF-□, eotaxin, IP-10, PP, VEGF
- **Manual Procedures**: HCG (urine or blood), occult blood, urine color, urine appearance,
- **Microscopic analyses (urine)**: amorphous, bacteria, casts, crystals, epithelial cells, mucus, red blood cells, white blood cells.
- **Perkin Elmer Wizard 2470 gamma counter (radioimmunoassay)**: 1,25-dihydroxy vitamin D, 17-hydroxyprogesterone, 25-hydroxy vitamin D, adiponectin, HMW adiponectin, aldosterone, angiotensin-1, DHEA, estrone sulfate, glucagon, leptin, melatonin, neuropeptide-Y, PINP, PYY, renin, resistin, reverse T3, PINP, calcitonin, calcitonin gene related peptide, CCK, CRF, total and active ghrelin
- **Quintron Breath Tracker**: breath hydrogen and methane gases.
- **Varian 240Z Atomic Absorption**: cadmium, copper, selenium
- **Perkin Elmer LS50B Luminometer**: ORAC, enterostatin, GLP-1
- **BiositeTriage Meter Pro**: urine drug screen, (amphetamine, barbiturate, benzodiazepine, cocaine, methadone, methamphetamine, morphine, phencyclidine, THC, tricyclic antidepressants)

- **Yellow Springs Instruments Glucose Analyzer**: glucose (point of care testing)

Mass Spectrometry Core
The Core is directed by Jennifer Rood, Ph.D., DABCC, FACB and is divided into two sections: Energy Expenditure/Body Composition and Metabolism

Energy Expenditure/Body Composition
This section focuses on the measurement of energy expenditure using the doubly labeled water technique. Additionally, measurements of total body water are performed using either deuterium or oxygen 18. This section has six Finnigan isotope ratio mass spectrometers (a Delta S, a Delta XP, two Delta Vs and two MAT 252s). The laboratory also has automated sample preparation devices interfaced to the mass spectrometers. Four gas benches are used for 18O sample preparation and five H devices are used for the sample preparation of deuterium (2H). With these instruments, we can accurately and precisely measure the amount of heavy isotopes, such as 18O and deuterium, in relation to the common isotopes, 16O and 1H, for the measurement of energy expenditure in studies of obesity. The instruments are also used to measure 18O and deuterium as measures of total body water. The Delta XP is also used for analysis of 13C in breath samples as a marker of gastric motility.

Metabolism
This section focuses on the measurement of stable isotopes that are used to examine lipid, protein, and carbohydrate metabolism. This section has three gas chromatograph/mass spectrometers (Agilent 6890 GC/5975 MS, Agilent 6890 GC/ 5975b MS and an Agilent 7890GC/5975c MS). All three mass spectrometers have EI and CI capabilities, and positive or negative ion monitoring, for measurement of any stable isotope labeled (e.g. 2H, 15N, 13C) organic compound. This equipment is used to examine cholesterol metabolism in studies of cardiovascular disease, and glucose, amino acid and fatty acid metabolism in studies of obesity and diabetes.

Nutritional Epidemiology, Dietary Assessment and Counseling Core
Directed by Catherine M. Champagne, PhD, RDN, LDN, FADA, the Nutritional Epidemiology Diet Assessment and Counseling Core serves two main needs at the Pennington Center: 1) processing of dietary data collected via food frequency questionnaires, 24-hour dietary recalls, or food records and 2) delivery of lifestyle interventions which follow defined protocols via single site or multi-center trials. The MENu Database, is overseen by Catherine Champagne. The MENu database was donated to the Pennington Biomedical Foundation in October, 1992, by its owner and developer, Dr. Margaret C. Moore. The Extended Table of Nutrient Values was renamed to honor the name of its developer. The Moore Extended Nutrient Database, now known as the MENu Database, is an appropriate reflection of one of its current uses in analyzing menus and recipes for the PBRC metabolic kitchen, for school lunches in Louisiana, and for multicenter feeding trials. The MENu Database was selected for use in the National Heart, Lung and Blood Institute multi-center study of diet and lipoproteins. When compared to analytical laboratory values obtained from an outside Food Composition Laboratory, the MENu Database was closer to actual values

than the three other databases used to calculate the same menus. Current data from additional menus analyzed still indicates good agreement between values from the MENu Database and the laboratory assays.

DIET ASSESSMENT ACTIVITIES:

The current version of Moore's Extended Nutrient Database (MENu) is MENu 6 (2005). Primary datasets used are from USDA. The total count of foods and recipes contained within the MENu food composition files comes from the following data sources:

- Release 26 of the USDA Nutrient Database for Standard Reference (October 2013).
- The Food and Nutrient Database for Dietary Studies 5.0 (March 2012) which is used to conduct the What We Eat in America dietary portion of NHANES, 2009-2010 (Ahuja JKA, Montville JB, Omolewa-Tomobi G, Heendeniya KY, Martin CL, Steinfeldt LC, Anand J, Adler ME, LaComb RP, and Moshfegh AJ. 2012. USDA Food and Nutrient Database for Dietary Studies, 5.0. U.S. Department of Agriculture, Agricultural Research Service, Food Surveys Research Group, Beltsville, MD).
- Supplementary information from the scientific literature or other reliable food composition tables.
- User defined foods, allowing the input of nutrient data for foods needed in menus or recipes for which an appropriate food match cannot be found otherwise.
- Recipes input by users of the system at PBRC, using a unique recipe calculation system.

Analysis of dietary intakes of individuals using the Food Diary Program. While menu and recipe analysis is an important activity using the MENu system, several current research protocols use the Food Diary Program. Food Diary utilizes the MENu 6 Food Composition Files to analyze dietary intakes of individuals in research studies. Often these are dietary records kept by participants in various trials or 24-hour recalls collected by the nutrition staff in the Dietary Assessment Center.

Food Frequency Questionnaires. In association with most major research projects involving collection of dietary intake data by food records, a number of studies also include the administration of food frequency questionnaires to capture intakes over a longer period of time. Pennington Biomedical Research Center uses the Food Frequency Questionnaire originally developed by Drs. Gladys Block and Linda Harlan, often referred to as the "Block FFQ". This questionnaire collects information about an individual's eating habits over a 12-month period, however it has often been modified to specific periods of time, e.g. over the last six months, during your pregnancy, etc., depending on the specific needs of the study. The FFQ contains approximately 105 items grouped by categories and is completed for both frequency of consumption as well as portion size selections by the individual. This is a scannable questionnaire with coding and file locations of the variables set by the DIETSYS technical support staff of the National Cancer Institute in conjunction with National Computer Systems, Inc (NCS) personnel. The questionnaire, upon completion, provides estimated daily intake values for selected nutrients (kilocalories, macronutrients, and micronutrients) and provides

information on food group servings. The original Block FFQ form was modified by reformatting it to be printed on plain paper using a laser printer. Each printed copy of the form contains a barcoded identifier. The steps in FFQ processing are scanning, analyzing, importing results, and data extraction. The physical forms are translated into electronic data, using Optical Mark Recognition (OMR) using Remark Office OMR 6 and are saved as ASCII files (regular text files, can be opened in Notepad). Scanning only converts the filled-in bubbles to electronic information meaning that the data is also stored as "raw data." An FFQ Scanning Wizard application prepares the raw data for analysis and the DIETSYS application actually transforms the prepared raw data into useful results. After analysis, the results must be imported into a central database for storage and later retrieval. Each FFQ is assigned to a subject (identified by the SubjectID), a study (POUNDS LOST, Hart D, etc), and, optionally, a timepoint (Baseline, Visit 1, etc.).

Reference: Block G, Thompson FE, Hartman AM, Larkin FA, Guire KE. Comparison of two dietary questionnaires validated against multiple dietary records collected during a 1-year period. J Am Diet Assoc 1992;92:686-693.

24-Hour Dietary Recall Collection Using the USDA Automated Multiple Pass Method.
Catherine Champagne and her staff of dietary assessment personnel have been trained by USDA in the use of the Automated Multiple Pass Method (AMPM) and have used this in a number of trials, e.g. the POUNDS LOST Clinical Trial which was a macronutrient based weight loss study involving PBRC and Harvard. AMPM is a computerized method for collecting interviewer-administered 24-hour dietary recalls either in person or by telephone. It is a research-based, multiple-pass approach employing 5 steps designed to enhance complete and accurate food recall and reduce respondent burden. This method is currently used in "What We Eat in America", the dietary interview component of the National Health and Nutrition Examination Survey, and other research studies nationally.

Diet History Questionnaire from the National Cancer Institute. DHQ II is the current version of the questionnaire distributed by the NCI. PBRC has developed an online version of this questionnaire and with the Diet*Calc software developed by NCI can analyze files to interpret the DHQ data to provide nutrient and food group estimates.

DIETARY COUNSELING ACTIVITIES:
A number of projects at the Pennington Biomedical Research Center have involved dietary counseling efforts. The Diabetes Prevention Project Outcomes Study (DPPOS) is following individuals from DPP who have successfully made lifestyle changes. The Look AHEAD trial also focused on lifestyle changes in a population of diabetic individuals. The Weight Loss Maintenance (WLM) trial was designed to determine how weight loss achieved in an intensive 6 month initial phase of lifestyle change sessions was best sustained through a second phase, 30-month period of either personal contact or internet efforts. The POUNDS LOST trial utilized four different diet treatments varying in protein and fat to scientifically test these diets for weight loss effects. Subjects were asked to follow structured meal plans or exchange options in order to

adhere to the dietary targets. The research dietitians/interventionists played a key role in working with these participants by conducting both group and individual sessions utilizing nutrition information and behavior change messages (a landmark paper was published in the New England Journal of Medicine February 26, 2009). Additional projects included a lifestyle program for weight loss in cancer survivors, studies on a variety of low-fat and higher fat Mediterranean diet regimens, and community interventions focused on weight control and diabetes care. Current activities involve a behavioral weight loss program in very obese individuals, which is being compared with individuals who opt for bariatric surgery. In essence, the dietary counseling activities have been extensive and the team of interventionists involved have a breadth of experience in dietary interventions which include lifestyle/behavioral change. These interventionists have received significant training in motivational interviewing and theories of behavioral change.

Ingestive Behavior Laboratory

The Ingestive Behavior Laboratory (IBL) is under the direction of Corby K. Martin, Ph.D., a Licensed Clinical Psychologist in the State of Louisiana. The IBL specializes in the assessment of energy intake and has developed and validated methodology for use in free-living conditions and controlled laboratory settings. The IBL is able to assess subjective ratings of appetite using Visual Analogue Scales (VAS), which complement objective measures of food intake. VAS are commonly used to assess changes in appetite and satiety due to feeding paradigms, pharmaceutical compounds, or behavioral (lifestyle) interventions. The IBL also empirically evaluates the effect of behavioral (lifestyle) and pharmacological interventions on energy intake and energy expenditure. Finally, the IBL specializes in free-living assessment of energy expenditure by using a variety of accelerometer devices and doubly labeled water (in conjunction with the Mass Spectrometry Core).

The IBL includes three separate eating rooms that are each equipped with Universal Eating Monitors (Kissileff, Klingsberg, & Van Itallie, 1980). Universal Eating Monitors consist of a scale that is concealed in a table and connected to a computer that automatically records the weight of food removed (eaten) from a plate on top of the scale. Each table is covered with a tablecloth, and the participant is not acutely aware that food intake is being monitored. Universal Eating Monitors allow analyses of cumulative food intake throughout the course of the meal, and each of the eating rooms includes a desktop computer that participants use to rate their subjective levels of appetite with VAS. The Laboratory also includes a monitoring room that houses desktop computers and closed-circuit video equipment to record food intake behavior in the adjacent eating rooms. Lastly, the laboratory includes a taste testing area and prep area, which allows food intake to be quantified by weighing food before and after participants' meals.

In addition to laboratory-based endpoints, energy intake can be measured in cafeteria-based settings (e.g., school cafeterias, military dining facilities) using the Digital Photography of Foods Method. The laboratory also developed and validated a Smartphone-based approach called the Remote Food Photography Method, which

accurately measures energy and nutrient intake in near real-time in participants' natural environment. The IBL has collaborated with computer programmers in San Diego, CA, to collect Remote Food Photography Method data with a Smartphone Application, called the SmartIntake App. SmartIntake collects data with very little user and researcher burden. The application allows users to enter Price Look-Up (PLU) codes of produce, scan barcodes on food packages, and record voice or send text descriptions of foods images captured. These data are packaged together with the food images, sent to IBL staff in real-time, and are used by IBL registered dietitians to quantify energy and nutrient intake.

BEHAVIOURIAL COUNSELING

The Ingestive Behavior Laboratory is comprised of doctoral and master's level behavioral psychologists and registered dietitians. The intervention team has designed and implemented numerous lifestyle interventions that modify food intake and exercise levels. The team has extensive experience with clinic-based (face-to-face) interventions. The team also performs translational research and disseminates population-based interventions that are evaluated in studies that rely on cluster randomized designs. Moreover, they are on the cutting edge of developing and testing the efficacy of e-Health interventions that are delivered via communication technologies (e.g., internet, Smartphone). The laboratory and its colleagues have also developed methods to objectively quantify adherence to dietary interventions based on observed body weight. These methods have been used successfully in NIH trials. Finally, the laboratory has developed screening paradigms and retention strategies to minimize attrition during randomized controlled trials.

Psychological Assessment Laboratory

The Psychological Assessment Laboratory (PAL) is under the direction of Robert Newton, Ph.D. The PAL is responsible for data collection and data management of psychological and behavioral questionnaires. The PAL creates and prints paper questionnaire packets, scans and scores completed questionnaires, manages scoring algorithms of questionnaires, and imports data to a database. The PAL works in conjunction with Computing Services to set up scoring algorithms and ensure the proper upload of data.

Research Kitchen and Food Preparation

The PBRC metabolic kitchen is located on the second floor of the Clinical Research Building No. 1 and has 2,622 SF of working space. The kitchen area is divided into four fully-equipped individual kitchen areas, each comprising 130 SF. These individual kitchens are ideal for simultaneously conducting various protocols. Each individual kitchen area is equipped with a refrigerator, freezer, microwave, cook-top, , one-quart blender, toaster, and electronic balances. There are several different models of electronic balances to accommodate weighing demands. One kitchen area is set up as a bake area, containing a 20-quart mixer. The 440 SF large quantity food preparation area contains state of the art convection ovens, steam ovens and kettle, bake ovens and cook-tops, microwave, food warmers, food chopper, slicer, food processor, one-gallon blender, and a large capacity electronic balance. There is a tray service area, a

dish room, and approximately 800 SF for receiving and storage including dry storage, a walk-in refrigerator, and a walk-in freezer. Located just outside of the Research Kitchen is the participant dining space. This includes a reception desk where meal trays can be requested by phone or buzzer. Meals that are taken for later consumption are stored in the refrigerator/freezer room. An additional food storage area with space for dry storage, refrigeration and freezer storage is located adjacent to the service road. Approximately 225 meals per day can be prepared in the facility. Study participants consume their meals in the 850 SF dining area, and meals may be provided for take-out.

The PBRC Research Kitchen is under the direction and supervision of Courtney Brock RD, LDN. The Research Kitchen has the capability of providing participants with all of their meals, seven days a week, in both inpatient and outpatient studies. Menus consisting of specific macronutrient & micronutrient composition or incorporating study-specific food products are developed to meet the needs of the study. The Research Kitchen has the infrastructure to observe and document consumption of study foods by participants seven days a week improving adherence to study regimens.

The Research Kitchen employs a Director who oversees menu planning, food production, and daily management of the operation. The Research Kitchen Manager is responsible for procuring foods, equipment, paper supplies, and other products necessary for study specific criteria. The Research Dietitians are responsible for managing the dietary component of specific study protocols. Research specialists and a food service worker prepare and serve the research-designated diets. Meal monitors and Hostesses sit with participants during meal time to ensure that participants are being compliant. Specified food products, if needed, are developed by the metabolic kitchen staff. Recipes are selected to include regional food preferences to increase dietary adherence. After taste-testing, the food products can be analyzed for nutrient content, and then included in the database for menu planning. The research dietitian reviews the finalized menu with each potential participant before the research project begins. The participant's food likes, dislikes and intolerances, including food allergies, are discussed. Food purchases are based on specifications outlined during the menu development to meet nutrient content requirements. Upon receiving, the product is inspected by the manager to ensure proper quality and weight specifications. When possible, foods to be used throughout the research study are purchased at one time from a single lot to ensure minimum variation, and are stored properly. Bulk food deliveries are stored in adjacent cold, dry or freezer space. Standardized recipes outlining specific ingredients and gram weights, correct mixing and cooking procedures, timing, and use of equipment are meticulously followed under sanitary procedures. All ingredients are weighted to 0.1 gram on electronic balances. Mixed foods are prepared in batch quantities. Those foods then are individually portioned, weighed, sealed, labeled, and frozen until ready to use. The nutrient composition of every study diet may be verified by chemical analysis of aliquots of each menu cycle to ensure that the designed menus achieve the target nutrient values predicted by the nutrient database. A continuous quality assurance program is followed to check food item weights, recipe procedures, packed meal and tray assembly, and food temperatures. Documentation is

maintained for each study. Furthermore, operational problems are documented with an appropriate plan of action and follow-up. All Research Kitchen staff members receive training in food sanitation and in research diet preparation.

Daily food production sheets for each participant are used when preparing the meals, listing day, menu cycle, food items required with portion weights, and special dietary requirements. Foods are labeled for participant identification. Foods are placed on individual meal trays until service, or are individually packaged for take-out, following tray assembly forms. Meals are served to the participant on test days only after all study procedures have been completed. Additionally, Research Kitchen staff obtains daily checklists from participants which contain information on the participant's consumption of meals provided. Potential problems with meal acceptance are identified and resolved. Personal attention and encouragement to continue on the diets are provided by all staff members throughout the study.

Energy Metabolism Core Laboratory

The energy metabolism core consists of four Respiration Chambers (whole-room indirect calorimeters) for the assessment of 12 and 24 hour energy expenditure and substrate oxidation, and nine portable ventilated hood systems (7 MaxII, AEI Technologies, Naperville, IL; 2 Deltatrac Metabolic Monitors, Datex-Ohmeda) for the assessment of resting energy expenditure and substrate oxidation and the thermic effect of feeding. The respiration chambers are located in the inpatient area of Clinical Research Building No. 2. Three of the chambers measure 14'5" x 10'7" with 8' ceilings corresponding to a total volume of about 27000 L. The fourth chamber is 9'7" x 8'5" for a total volume of 16500 L. Each chamber is provided with furnishings and equipment necessary to perform metabolic studies on research volunteers over extended time periods in a precisely controlled environment. The three large chambers are comfortable enough for individuals to live for periods up to one week. They are equipped with a bed, chair, desk, toilet, sink, refrigerator, TV, VCR/DVD, computer with internet access, treadmill, and motion sensors. The smaller chamber is equipped with a treadmill, roll away bed, chair, and is used for short term energy expenditure testing. The respiration chambers and the ventilated hood systems utilize air that is drawn through the unit at a known flow rate. The oxygen and carbon dioxide concentrations of incoming outgoing air are measured for the calculation of oxygen consumption and carbon dioxide production from which energy expenditure is calculated from the Weir equation. If nitrogen excretion in urine is also measured, substrate oxidation rate can be calculated as well. Eric Ravussin, Ph.D. is the scientific director and Leanne Redman, Ph.D. oversees the data integrity. In addition, the unit employs a biomedical engineer to ensure accurate data collection and on-going maintenance and calibration of the equipment. Volunteer testing is performed by trained staff members.

Current services include but are not limited to:

- 12 and 24 hour energy expenditure and substrate oxidation
- Resting energy expenditure and substrate oxidation under basal conditions and during a euglycemic hyperinsulinemic clamp
- Data analysis with correction for urinary nitrogen

- Thermic effect of food

Imaging Core

The Imaging Core is designed to provide in vivo measurements of anatomy, biochemistry, metabolism, and tissue function for clinical research. Key Imaging Core instruments include two 3T MRI machines (a 70cm-bore GE Discovery 750 and 60cm-bore GE Signa HDxT), two DXA instruments (GE Lunar iDXA and Hologic Discovery), two ECHO MRI [ultra-low field strength NMR] instruments (one designed for adults and one designed for infants), BodPod and PeaPod systems by Cosmed, and two ultrasound devices (Toshiba Aplio SSA-770 and GE LogiqE9). A GE diagnostic X-Ray system was also recently installed. Researchers also have access to a 64-slice CT scanner and PET scanners through collaborative agreements with Baton Rouge General Hospital– Bluebonnet campus and the Mary Bird Perkins Cancer Center.

Both MRI systems have full multinuclear spectroscopy capabilities (31P, 13C, and 1H RF and a proton decoupler). A spectrum of coils is available for spectroscopy (4cm, 6cm, and 8 cm singly and doubly tuned 31P transmit/receive), as well as standard pulse sequences. Spectroscopy analysis software (jMRUI) is installed on workstations in the scanner suites.

An array of coils is also available on both systems for volumetric imaging including head, head-neck, body, spine, and knee boils. A 32-channel phased array head coil is also available on the Discovery system for brain applications. A set of volumetric imaging sequences are available for body composition including the IDEAL-IQ water-fat sequence on the Discovery and LAVA on both systems. The increased bore size (70 cm) of the Discovery system and the resulting increased field of view (50cm x 50cm x 50cm) allows the Core to accommodate larger subjects for all scan types. Standard T1-, T2-, T2*-, perfusion-, and diffusion-weighted sequences are available for brain applications. Specific state of the art sequences of interest include high-angular-resolution diffusion imaging (HARDI), echo planar imaging with blood oxygenation level dependent contrast (EPI-BOLD), partial continuous arterial spin labeling (PCASL) for perfusion imaging, and fluid attenuated inversion recovery (FLAIR). Visual and audio stimulus presentation hardware and software are available for fMRI studies. Image analysis software includes Analyze (Analyze Direct), a suite of structural brain imaging tools originally developed in the IDEA Lab at UC Davis, and SPM and FSL for structural and functional MRI analysis.

Active, optimized research protocols include the following:

- 1H (proton) spectroscopy for the measurement of hepatic lipid
- 1H (proton) spectroscopy for the measurement of intramyocellular lipid
- LAVA and IDEAL-IQ imaging for measurement of adipose tissue, muscle, and bone abdominally and throughout the body
- 31P mitochondrial phosphorylation capacity (ATPmax)
- 31P resting ATPase measured pre-post lower leg ischemia
- LAVA-based measurement of internal organ volumes
- Brain tissue structure using T1-weighted and FLAIR imaging

- Cerebral blood flow and brain activation by PCASL and EPI-BOLD.

The Biomedical Imaging Center includes two subject waiting rooms, two subject dressing rooms, and two subject preparation rooms. The Center also contains office space for researchers and administrative staff, reception and waiting areas, three registration desks, two conference rooms, a large medical records storage room, computer and data closets, and an open-plan meeting and social area.

Core personnel currently includes 3 licensed radiological technologists (an MR technologist, a CT technologist, and a DXA technologist), an ultrasound technologist, and a biomedical engineer and core manager (M.S.). Each of these individuals are cross-trained to perform analyses of MRI data; ultrasound data analyses are performed in-house by the ultrasound technologist as well. Baton Rouge Radiology Group serves as a consultant for interpretation of scans as needed. Analyzed data is entered into a centralized database where it is fused with other participant records such analyzed on-site and is directly transferred to the Pennington Biomedical Research Center database.

Exercise Testing Core
The Exercise Testing Core is directed by Jennifer Rood, PhD and is equipped to perform electrocardiogram (ECG) monitored sub-maximal and maximal cardiovascular exercise performance testing and musculoskeletal strength and endurance assessments. The Core includes two Parvomedics True One™ metabolic carts for the performance of metabolic testing (i.e. VO2), two Trackmaster treadmills TMX425C and a Lode Excalibur Sport™ bicycle ergometer. The Valiant Treadmill has a zero starting speed and acceleration capabilities ranging from 0.2 - 40 km/h (0.125-25Mph) and is capable of imposing both positive (+25%) and negative (-10%) inclines, while accommodating patients weight up to 340 kg (750 lbs). The Lode Excalibur Sport ergometer is capable of singular wattage increments ranging from 0-1000 W. The Core has 2 ECG machines including a Quinton Q-Stress and newly acquired wireless Mortara X-Scribe system.

The Core also includes a Biodex™ isokinetic strength dynamometer to perform constant velocity muscular strength and endurance testing. The Biodex system interfaces with computer microprocessors to measure torque, power, and endurance for resistance throughout a joint's range of motion (ROM) of most musculoskeletal joint areas. Resistance is provided using a servo-controlled mechanism and a user-defined constant velocity.

PBRC Pharmacy Operations
The pharmacy is operated by Claire Hazlett, RPh. When a study medication arrives at the Center, the shipment is verified by the pharmacist and all records are kept according to each specific study protocol. If a study medication arrives in bulk form, the pharmacist is responsible for counting and labels the medication in the study-appropriate fashion before it is dispensed to study subjects. An accountability form is completed when a study medication is packaged to keep a record of which subjects received placebo or active. This form records the amount of medication remaining in

the pharmacy as well as expiration dates. Accurate accounting for the dispensing of a study drug is maintained by the pharmacist. When a drug leaves the pharmacy for dispensing to a subject, the study coordinator is required to complete a dispensing log. This record requires documentation of: the subject identification number, subject initials, coordinator who dispensed, number of bottles/tablets dispensed, date dispensed and if application the amount of drug returned. When study medication is ready for distribution, it will be transported by the pharmacist to the locked pharmacy storage area on the ground floor of Clinic Building 1.

The pharmacy can compound non-sterile products into different dosage forms including capsules, liquids and nasal sprays. The pharmacy is equipped with a clean room where sterile compounds can be prepared.