TITLE: OPTIMIZING PROTEIN INTAKE IN OLDER AMERICANS WITH MOBILITY LIMITATIONS

THE STUDY PROTOCOL

PRINCIPAL INVESTIGATOR: SHALENDER BHASIN, MD

JOINT PI: CAROLINE APOVIAN, MD

CO-INVESTIGATORS: LYNN MOORE, TOM TRAVISON, THOMAS W. STORER, SHEHZAD BASARIA, ALAN JETTE

FUNDING: NATIONAL INSTITUTE ON AGING

NIA GRANT NUMBER: 1R01AG037547-01

APPLICANT ORGANIZATION: BRIGHAM AND WOMEN'S HOSPITAL

VERSION: 5.3 **DATE: 08/23/2016**

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Study Summary

Title	Optimizing Protein Intake in Older Americans (OPTIMen)
Protocol Number	1R01AG037547-01
Study Design	Randomized, placebo-controlled, parallel group, double-blind
Study Duration	Five years
Study Center	Single site: Brigham and Women's Hospital
Objectives	To determine whether administration of 1.3 g • kg^{-1} • day^{-1} of protein results in greater improvements than the RDA (0.8 g • kg^{-1} • day^{-1}) in lean body mass, maximal voluntary muscle strength and power, and self-reported and performance-based measures of physical function in older men whose daily protein intake is less than the RDA and in whom energy intake has been standardized at 30 kcal• kg^{-1} • day^{-1}
Number of Subjects	152
Main Inclusion Criteria	Community dwelling, older men, 65 years of age or older, who have SPPB scores between 3 and 10, and protein intake <0.83/g/day
Study Product, Dose, Route, Regimen	Testosterone enanthate in sesame oil 100 mg intramuscularly weekly
Duration of administration	6 months
Statistical Methodology	Our primary outcome is change in lean body mass from baseline to 6 months assessed by using dual energy X-ray absorptiometry (DXA). Our primary analytical strategy is to use mixed-effects regression analysis to assess 3 and 6 month outcomes simultaneously with baseline total body lean mass considered as a covariate, as is appropriate for between-group comparisons of randomized subcohorts, and 6-month differences between arms will be estimated via a treatment contrast and corresponding 95% confidence interval.

1. Study Objectives

The recommended dietary allowance (RDA) for protein has been set at 0.8 grams/kg/day; this allowance is intended to meet the needs of essentially the entire adult population – men and women, young and old (1). However, the protein requirements in older individuals remain poorly validated and have engendered vigorous debate (2-6). Many experts advocate dietary protein intakes substantially above the current RDA to help maintain muscle anabolism in older individuals; these scientists cite epidemiological studies and intervention trials which suggest that protein intakes above the current RDA (0.8 g • kg^{-1} • day^{-1}) for older adults have beneficial anabolic effects on the skeletal muscle (2, 3, 7-17). Other experts have expressed strong dissenting views and support the current protein RDA (4, 5). The efficacy of protein intakes in excess of the RDA in improving skeletal muscle mass, muscle performance and physical function has not been demonstrated in older individuals and is an issue of enormous public health and policy implications.

An exhaustive survey of nutrient intakes in approximately 50,000 Americans residing in 48 states by the US Department of Agriculture revealed that about a third of older Americans do not ingest the RDA for protein (18); more recent surveys have confirmed these findings (19). Low protein intake has been implicated as a remediable contributor to the multifactorial pathophysiology of sarcopenia, the aging-associated loss of muscle mass and function, which has been recognized by the Centers for Disease Control and National Institute on Aging as one of the major health risks facing older Americans (2, 7, 9, 11, 13-15). However, we do not know whether increasing protein intake in older Americans, whose protein intake is below the RDA, increases skeletal muscle mass, muscle performance and physical function. Therefore, the **primary objective** of this investigation is to determine whether increasing protein intake in older Americans with daily protein intake less than the RDA to 1.3 g • kg^{-1} • day^{-1} will result in greater gains in lean body mass, muscle performance and physical function than protein intake that equals the RDA (0.8 g • kg^{-1} • day^{-1}).

Nitrogen balance studies in athletes have suggested that protein intake substantially greater than the RDA may be required to maintain nitrogen balance in individuals undergoing resistance exercise training and that even higher protein intake may be needed to achieve positive nitrogen balance and optimize adaptations to exercise training in these athletes. Remarkably, however, the studies in older individuals have failed to demonstrate unequivocal improvements in lean body mass or physical function in older individuals fed higher amounts of protein in excess of the RDA. The protein requirements for achieving optimal anabolic response to administration of promyogenic function promoting therapies are unknown. Therefore, our **second objective** is to determine whether a higher level of protein intake - in excess of the RDA - is needed in older individuals to optimize anabolic response to a promyogenic function promoting anabolic therapy.

Specific Aim 1

To determine whether administration of 1.3 g • kg^{-1} • day^{-1} of protein results in greater improvements than the RDA (0.8 g • kg^{-1} • day^{-1}) in lean body mass, maximal

voluntary muscle strength and power, and self-reported and performance-based measures of physical function in older men whose daily protein intake is less than the RDA and in whom energy intake has been standardized at 30 kcal• kg^{-1} • day^{-1}

Hypothesis 1

In older individuals whose daily protein intake is less than the RDA, increasing dietary protein intake to 1.3 g • kg^{-1} • day^{-1} without changing the total daily energy intake, will not result in greater improvements in lean body mass, maximal voluntary strength, and self-reported as well as performance-based measures of physical function than ingesting the RDA.

Specific Aim 2

To determine whether the gains in lean body mass, maximal voluntary strength, and self-reported and performance-based measures of physical function during testosterone administration are greater with 1.3 g protein than the RDA in older men whose baseline daily protein intake is less than or equal to the RDA and in whom energy intake has been standardized

Hypothesis 2

The RDA of 0.8 g • kg^{-1} • day^{-1} protein intake is insufficient to attain optimal anabolic response to administration of a promyogenic function promoting therapy, such as testosterone. In individuals whose daily protein intake is less than the RDA, increasing dietary protein intake to 1.3 g • kg^{-1} • day^{-1} without changing the total daily energy intake, will augment anabolic response to testosterone therapy and will be associated with greater gains in lean body mass and maximal voluntary strength than the RDA.

2. Introduction

This document is a protocol for a human research study to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and institutional research policies and procedures.

2.1. Background

Protein requirements for older individuals have been the subject of contentious debate for some time. Currently, the Estimated Average Requirement (EAR) for protein (0.66 g protein • kg body $weight^{-1}$• day^{-1}) and the Recommended Dietary Allowance (RDA) for protein are the same for adult men and women of all ages (1, 20). While the RDAs have assumed the force of biblical diction and guided national policy and clinical practice, the data on which the RDA for protein is founded have been justifiably criticized (2, 3, 10, 17, 21). The RDA estimate has been derived largely from short term studies of two to three week duration, using nitrogen balance as the outcome (1, 20). Numerous conceptual and methodological issues related to nitrogen balance studies (1, 3), and additional issues related to subject selection have clouded the inferences drawn from these studies (3, 10). Most of the original nitrogen balance studies that formed the

basis of these recommendations were conducted after only 2-3 weeks of adaptation (4, 20); urea turnover is slow and these short durations were likely insufficient to achieve a metabolic steady state (10). The nitrogen balance studies generally used low levels of protein intake; adaptation to this low level of protein intake would be expected to reduce urinary nitrogen excretion. Thus, the calculation of protein level required to achieve zero nitrogen balance would likely be underestimated. These studies included only a small number of older men and women. None of the original studies included measurements of lean body mass, muscle performance or physical function (1). The nitrogen balance studies have been criticized on multiple other grounds: inclusion of very few older individuals, inclusion of subjects with undocumented medical conditions, problems with complete urine and fecal collections, not accounting for other sources of nitrogen loss, and inclusion of subjects with inadequate or excessive energy intakes (10). Not surprisingly, the recommendations for older individuals have invited a great deal of disagreement; some experts have advocated higher levels of protein intake for older individuals (2, 6, 10, 21-27), while others have equally fervently supported the adequacy of the current RDA (4, 5, 20-21, 28-29). Thus, the issue being addressed in this investigation has enormous public health and policy implications: if the protein requirements to maintain optimum muscle anabolism are higher for older individuals than young persons - as has been advocated by some - then a reconsideration of public policy and clinical practice is warranted. As a vast majority of older Americans ingest substantially lower amounts of protein than those being recommended by some (30-32), implementation of a change in protein RDA for older Americans will not be easy.

The proposed investigation also has implications for the application of pharmacological function promoting anabolic therapies (FPATs), such as testosterone, selective androgen receptor modulators (SARMs), recombinant human growth hormone, growth hormone secretagogs, and myostatin antagonists. We do not know whether protein intakes higher than the RDA are needed to realize optimal anabolic response to FPATs in older individuals. Thus, the proposed study will help determine whether protein intake higher than the RDA augments the anabolic response of older individuals to a prototypical pharmacologic anabolic intervention, namely testosterone, whose effects on skeletal muscle mass and strength have been well documented.

2.1.2. Functional Limitations and Sarcopenia in Older Americans as Major Societal Problems

As men and women get older, skeletal muscle mass declines because of a decrease in the number and cross sectional area of muscle fibers (33-37) and a preferential loss of fast twitch type II fibers (38-39). The loss of muscle mass that occurs with aging is associated with a reduction in muscle strength and power, and an increase in muscle fatigability (36, 40-45). Loss of muscle mass and strength leads to impairment of physical function, as indicated by the impaired ability to rise from a chair, climb stairs, generate gait speed, and maintain balance (46-47). Limitations in physical function are associated with adverse health outcomes: increased risk of disability, mortality, hospitalization, and poor quality of life (48-59).

The 2003 National Health Interview Survey and 2004 US Census Bureau estimate that 12–14% of non-institutionalized population—approx. 35–38 million people—have a

disability due to a chronic health condition (60). For people over age 65, 35 to 40% experience activity limitations or disability. Because U.S. population is aging, the percentage of population aged 65 years or over will increase from 12% in 2000 to 20% in 2030—to over 69 million (61-63). The number of people aged 85 or over is expected to grow from 3 million (2.1%) to 6.2 million (3.4%). The majority of individuals who reach this age will experience some limitation in function. If the rate of limitations for those > 65 were to stay at 40% through 2030, the number of older people with functional limitations or disability would increase to 28 million (63). The costs of support services and lost productivity associated with disabling conditions have consequences for the entire population (58, 64). The Social Security Disability Program and the Supplemental Security Program pay $62.5 billion yearly to 7.5 million disabled persons (63). Thus, there is a pressing need for function promoting anabolic therapies – nutritional, physical, as well as pharmacological.

2.1.3. Multifactorial Pathophysiology of Aging-Associated Sarcopenia

Functional limitations associated with aging are multifactorial in their pathophysiology; impaired protein and energy intake, androgen deficiency, abnormalities in the growth hormone-insulin like growth factor-1 axis, anabolic resistance, inflammation, motor neuron remodeling, perturbations of muscle protein dynamics, and decreased physical activity are all likely contributors (12, 17, 65, 66-73). Of these factors, inadequate dietary protein intake has been well documented to accelerate this process (12-14, 17, 74), is potentially reversible, and is the focus of this proposal.

2.1.4. High prevalence of suboptimal protein intake in older Americans

A USDA survey in the late 1970s revealed that a third of the US population of 48 states consumed less protein than the RDA (30). More recent data from the 1996 Continuing Survey of Food Intake by Individuals largely confirmed the findings of the 1978 survey that ~40% of men and women, 70 years of age or older, consume less than the RDA for protein (0.8 g · kg^{-1} · d^{-1}), and ~16% consumed less than 75% of the RDA (31). In addition, older adults consume inadequate amounts of good quality animal protein.

2.1.5. Epidemiological studies of the health consequences of low protein diet

A few cross-sectional studies that have examined the association of dietary protein intake and body composition in older adults have not found an association between protein intake with lean body mass (34, 75). In one of the most comprehensive analyses of the longitudinal data from the Health ABC Study, Houston et al (14) found that dietary protein intake was associated with changes in lean body mass during a 3-year follow-up period in older, community-dwelling adults. Participants in the highest quintile of energy-adjusted protein intake lost ~40% less lean mass and appendicular skeletal muscle mass over the 3-year follow-up than those in the lowest quintile (14). Among individuals, who lost weight over the 3-year period, lower protein intake was associated with greater loss of lean body mass (14). A 10-year longitudinal study in New Mexico reported that women with protein intakes of 1.76 g protein • kg^{-1} • day^{-1} had fewer health problems than those consuming the RDA (76).

2.1.6. Intervention trials of varying levels of protein intake

Several small, short term studies of 9-14 week duration have examined the effects of alterations in protein intake on body composition and nitrogen balance (25, 77-78). Castaneda *et al* (79) found that older women fed a weight-maintenance diet with low protein content remained in a negative nitrogen balance throughout the 9-week study, and experienced significant losses in lean body mass, immune response, and muscle function, and muscle fiber atrophy (78). In another diet study (11), ambulatory men and women, aged 55 to 77 years, who were provided eucaloric diets that contained 0.8 g protein·kg^{-1}·day^{-1}, experienced significant loss of mid-thigh muscle mass and urinary nitrogen excretion.

Most studies that have compared the effects of high protein intake with the protein RDA have failed to find significant gains in lean body mass or muscle strength with the higher protein intake in spite of positive nitrogen balance with the higher protein intake (23, 80-82). A meta-analysis of trials that investigated the effects of increased protein and energy intake on health outcomes found a small weight gain but no improvements in function or mortality (83) in subjects receiving the supplement; this meta-analysis has been criticized for including heterogeneous trials in widely divergent populations, including hospitalized patients. A long term randomized trial of high protein-energy supplementation in frail elderly also found no difference in lean body mass between the placebo and protein-supplemented groups (84). Amino acid supplementation in some small studies has been shown to modestly increase muscle protein synthesis and lean body mass (85-87). Thus, overall, the evidence supporting the assertion that increasing protein intake above the RDA increases lean body mass, strength or physical function is weak.

The reasons for the striking failure of high protein intake alone to increase lean body mass in older individuals are not entirely clear, but several explanations are plausible. Most studies of high protein intake were of short duration and longer duration may be needed to accrue new muscle mass. Fiatarone et al (81) found that older subjects who were fed a nutritional supplement reduced their intake of other foods; therefore, the net protein-energy intake remained unchanged. Volpi (88), Rennie (80, 89), and others (106, 107) have invoked an intrinsic anabolic resistance in older individuals (65, 80, 88) due to age-related deficits in protein kinase B/ mTOR/ eIF4BP1 signaling pathways (90), rendering them unable to assimilate the increased protein intake into skeletal muscle mass accretion. This anabolic resistance may be overcome by resistance exercise training or by other anabolic stimuli (80, 91). Indeed, protein intakes in excess of RDA, when administered in conjunction with an anabolic stimulus, such as resistance exercise training or testosterone, have been associated with significant gains in lean body mass and muscle strength (81, 92). Trials conducted in athletes or young volunteers (8, 94-96) suggest that higher protein intakes enhance skeletal muscle mass and strength gains during resistance exercise training leading the International Society of Sports Nutrition to recommend in a position statement that "*protein intakes of 1.4 – 2.0 g • kg^{-1} • day^{-1} for physically active individuals are not only safe, but may improve the training adaptations to exercise training (8)*." Taken together, these data led us to hypothesize that increasing dietary protein alone is insufficient to induce lean mass accretion, but that higher protein intake, given in the setting of an anabolic therapy such

as testosterone or resistance exercise training, would be associated with significant gains in lean body mass, muscle strength, and physical function.

2.1.7. Potential Adverse Effects of High Protein Diet

There are no long term, adequately powered studies to determine the adverse health consequences of eucaloric, high protein diets in older individuals. Potential risks include the risk of renal dysfunction, hypercalciurea, and osteoporosis. Additionally, people often perceive high protein diets as being "unhealthy" – containing "too much fat or animal products". For every 25-g increase in dietary protein, urinary calcium increases by 0.8 mmol (97). There is concern that increased calcium exertion during ingestion of high protein diet may reflect bone demineralization (98-99) and increase the risk of kidney stones (100). Increased amounts of dietary protein also might be expected to increase endogenous acid production (101) and bone may be called upon to buffer the additional acid load, resulting in mobilization of bone calcium, and a decline in bone mineral density (BMD). A few cross-sectional studies have reported a positive association between dietary protein intake and hip fracture incidence (102-103). However, bone balance studies have not found an increase in bone calcium resorption. Instead, Kerstetter et al (97) found that additional dietary protein promotes intestinal calcium absorption and reduces the fraction of urinary calcium of bone origin. Also at odds with the bone hypothesis are a large number of epidemiological studies showing that a high-protein diet is associated with high, not low BMD (98, 104-105). The net effect of dietary protein on bone mineral status depends on dietary availability of calcium (106); a diet rich in protein, dairy, and calcium attenuates urinary calcium and bone loss relative to a high carbohydrate diet (107).

Brenner had hypothesized that high protein intakes by diabetic individuals create hyperfiltration and glomerular hypertension resulting in renal damage (108); however, this hypothesis has not found strong support (109). There is no evidence that high protein intakes up to 2 g $\cdot kg^{-1} \cdot d^{-1}$ increase the risk of renal impairment in individuals with normal renal function (110-114). However, in individuals with pre-existing impairment of renal function, high protein intake greater than 45% of energy intake may accelerate the decline in renal function (110, 114). The amount of protein being investigated in this study (0.8 and 1.3 g $\cdot$ kg^{-1} $\cdot$ day^{-1}) is lower than the levels that have associated with renal impairment. Also, we will exclude individuals with estimated GFR <60 ml/min. We will monitor serum creatinine and 24-hour calcium excretion during the course of the study.

2.1.8. Enhancing Compliance with Dietary Macronutrient Interventions

Several approaches – packaged meals, portion control, frequent contact and reinforcements, and frequent checks of dietary compliance in real time by using food compliance checklists - have proven effective in enhancing compliance with dietary prescriptions in nutrition intervention trials. Packaged meals and portion control are widely acknowledged as important contributors to improved compliance (115-116). Numerous trials have established the value of packaged meals, portion control, frequent contact and reinforcements, and frequent checks of dietary compliance in real time by using compliance checklists in enhancing compliance with dietary and lifestyle

interventions. Accordingly, we have incorporated all of these strategies in the design of the proposed trial.

2.1.9. Effects of Testosterone on Muscle Mass, Muscle Performance, and Physical Function

Several lines of evidence suggest that the decrease in testosterone with increasing age could be a cause of the decrease in muscle mass and strength and the decrease in physical function with increasing age. The men who are hypogonadal due to pituitary or testicular disease or due to treatment with GnRH analogs have decreased muscle mass (17-119). In population studies, lower testosterone concentrations are associated with decreased muscle mass and strength (34-36, 120), and physical function, and increased risk of falls (121). In the Longitudinal Aging Study of Amsterdam and MrOS, serum testosterone concentrations were positively correlated with muscle strength and physical performance (120-121). In the Massachusetts Male Aging Study, performance on a physical function test was positively correlated with the serum testosterone concentration up to the threshold value of 461 ng/dL (122). Self-reported physical function, as assessed by the physical function domain of SF-36, has been associated with total and bioavailable testosterone levels (123). In the MMAS, both total and free T levels were lower in men considered frail by either slow walking or grip strength (124).

Testosterone treatment of young hypogonadal men who have testosterone deficiency significantly increases lean body mass (125-129) and muscle strength (125, 129). Testosterone trials in men who have low testosterone levels in association with HIV wasting (129-132) and chronic obstructive pulmonary disease (133) also demonstrate increases in lean body mass and muscle strength.

Meta-analyses of randomized controlled trials (134-135) in which older men with low or low-normal testosterone concentrations were treated with testosterone have demonstrated an increase in lean body mass (136-146), muscle strength, and self-reported physical function (129, 133, 147). The effects on performance-based measures of physical function have been inconsistent across trials (137, 143, 145-146). Many reasons have been postulated for the inconsistency in the effects of testosterone on physical function measures: inclusion of healthy men with no functional limitations, confounding due to the ceiling and training effects, and the use of relatively low doses of testosterone which failed to significantly raise testosterone trials in some trials.

In healthy older men, in whom endogenous testosterone production had been suppressed by administration of a GnRH agonist, graded doses of testosterone were associated with dose-dependent increments in appendicular skeletal muscle mass and leg press strength (92). Only limited data are available on the effects of testosterone therapy on mobility, as determined by walking speed. In a randomized clinical trial in which 70 men were randomized to receive testosterone enanthate intramuscularly with or without finasteride or to receive placebo for three years, the men treated with testosterone, in comparison to those who received placebo, experienced significantly greater increases in a composite timed test of physical function that included walking speed (139). In another study of patients with congestive heart failure, testosterone therapy was associated with greater increase in shuttle walk distance than placebo (150). In this study, the increments in walking distance were correlated with changes in

bio-T levels (150). A randomized SARM trial also found significant improvements in stair climbing power (151). Accordingly, we have emphasized measures of skeletal muscle mass, muscle performance, and physical function, which have been shown to be androgen responsive and which are important for activities of daily living and mobility (e.g., walking and stair climbing).

3. Study Design

This will be a randomized, placebo-controlled, parallel group, double blind**,** clinical trial in community dwelling, older men, 65 years of age or older, who have mobility limitation. The study will have a 2 X 2 factorial design, which will allow us to investigate the effects of dietary protein intake and testosterone separately and together.

4. Outcome Measures

4.1. Primary Outcome Measure

Our primary outcome is change in lean body mass, measured by dual energy X-ray absorptiometry (DXA), because lean body mass is an excellent marker of whole body protein anabolism, is responsive to testosterone administration, and can be measured accurately and precisely by DXA (152-153). At a population level, sarcopenia defined in terms of lean body mass is predictive of disability, fracture risk, and other outcomes (34-36, 154). We recognize that translation of lean body mass gains into muscle performance and physical function gains is crucial for establishing the efficacy of function promoting therapies. Accordingly, we have included measures of muscle performance and physical function.

4.2. Secondary Outcome Measures

Tests of Muscle Performance

i. Maximal voluntary strength measured by the 1-repetition maximum method in the leg press exercise

ii. In addition, we will measure maximal voluntary strength in the chest press exercise. This exercise was chosen because it involves the large muscle groups of the upper extremities.

iii. We will measure the power of hip and knee extension by Bassey's leg rig.

Tests of Physical Function and Task-Specific Performance

i. 6-minute walking distance and speed (which is a reciprocal of walking distance)

ii. Stair-climbing power and speed without and with 20% load carry

iii. 50-meter timed walk with 20% load carry

d. Self-Reported Physical Function. We will characterize self-reported physical function by using the physical function domain of the Medical Outcomes Study Short Form-36 (SF36). Systematic reviews of randomized trials have shown that testosterone

therapy improves self-reported physical function, as assessed by the physical function domain of SF-36.

e. Sense of well being will be assessed by Psychological Well Being Index, and fatigue by FACIT-1 Fatigue scale; these outcomes have been shown to be androgen responsive. Positive and negative affect will be assessed by Derogatis Affective Balance Scale (DABS).

Safety measures

In accordance with the recommendations of the Endocrine Society's Expert Panel for Testosterone Therapy of Androgen Deficiency Syndrome in Men (150), we will monitor the following to assure subject safety:

i. Hemoglobin and hematocrit

ii. Serum PSA levels, periodic digital rectal examinations of the prostate, and American Urological Association/ International Prostate Symptom Score (AUA/IPSS) score

iii. Plasma lipids and apolipoprotein levels

There is no evidence that testosterone enanthate, when administered by intramuscular injection at the proposed dose, affects liver enzymes. There is weak evidence linking testosterone therapy to obstructive sleep apnea; both worsening and improvements in sleep apnea have been reported with testosterone administration, leading the Endocrine Society expert panel not to recommend monitoring for sleep apnea. Although there is no evidence that protein intakes of up to 2 g• kg^{-1}• $day^{-1}$are associated with adverse health effects, we will monitor:

i. 24-hour urinary calcium excretion

ii. Blood urea nitrogen (BUN) and creatinine

iii. Blood chemistry panel, including albumin, prealbumin and transferrin as markers of protein status

This study is neither sufficiently long in duration nor is adequately powered to determine the effects on bone mineral density and fracture risk. Demonstration of the beneficial effects on muscle mass, muscle performance, and physical function in this trial may justify a larger and longer study.

5. Subject Selection and Withdrawal

5.1. Number of Subjects

We estimate that **152 men** will be needed to test the primary hypothesis, based on assumption of two-sided, type I error rate of 0.05 and an intent-to-treat analytical strategy. We assume that no more than 20% of subjects in four groups will drop out before end of study.

5.2. Inclusion Criteria

1. Community-dwelling men 65 years of age or older

2. A score of 3-10 on the short physical performance battery (SPPB). If score is 10, than combination must meet 4-3-3 (Balance, Walk, Chair stand)
3. Daily protein intake less than the recommended daily allowance of 0.83 g • kg^{-1} • day^{-1} (from the average of three 24-hour food recalls or below 0.83 on two out of three days)
4. Able to give informed consent

4.3. Exclusion Criteria

1) History of prostate or breast cancer
2) American Urological Association [AUA] symptom index score of > 19
3) Prostate specific antigen (PSA) > 3 ng/ml in Black men or > 4 ng/ml in men who are not Black (since PSA levels are inherently variable and show lot of fluctuation, PSA levels may be repeated once if the initial levels were close to our eligibility criterion)
4) PSA > 3 ng/ml in those with history of prostate cancer in first degree relatives. These subjects may be enrolled if they have a negative transrectal biopsy within the past year.
5) Myocardial infarction or stroke within the last 6 months
6) Uncontrolled congestive heart failure, based on the study physician's evaluation
7) A. Serum creatinine > 2.0 mg/dL

   B. Men on any kind of dialysis will be excluded.
8) History of celiac disease, Crohn's disease, or ulcerative colitis
9) History of any malignancy requiring treatment within the previous 2 years, except non-melanomic skin cancers. Men with cancers who have not required active treatment within the past two years and who have not had disease recurrence within the past two years may be enrolled at the discretion of the study physician.
10) Neuromuscular diseases: motor neuron diseases, multiple sclerosis, adult muscular dystrophies, and myasthenia gravis
11) History of stroke with residual limb weakness that affected the individual's ability to walk; subjects with history of stroke who do not have residual limb weakness may be enrolled.
12) Schizophrenia, bipolar disorder, or untreated diagnosed depression. Subjects with unipolar depression who are on an antidepressant medication are eligible.
13) TSH levels < 0.4 or > 7.5 mIU/L(Since TSH levels are inherently variable and show lot of fluctuation, TSH levels may be repeated once if the initial levels were close to our eligibility criterion)
14) Systolic blood pressure (BP) > 160 or diastolic BP > 100 mm Hg (average of 2 measurements taken at visit 1) (Blood pressure may be repeated at visit 2 if the participant forgot to take antihypertensive medications at visit 1. Blood pressure may also be repeated at visit 2 or if the participant had elevated blood pressure at visit 1 but is interested in participating in the trial and wants to speak with his PCP regarding medication adjustment).

15) A. Hemoglobin A1c > 8.0%

B. Subjects on insulin therapy will be excluded.

Men with diabetes mellitus whose A1C is less than 8.0% or who are not taking insulin will be eligible.

16) Mini-Mental Status Exam [MMSE] <24

17) Body mass index (BMI) less than 20 or greater than 40 kg/m^2

18) Vegans and strict vegetarians, who are not willing to eat red meat, fish, shellfish, poultry, or eggs

19) Allergy to peanuts, soy, sesame, shellfish, or gluten

20) Current alcohol use >21 drinks/week based on self-report

21) Confinement to a wheelchair

22) Current use of any of the following medications:

a) Use of other anabolic therapies: testosterone, DHEA and androstendione, or rhGH within 1 year of screening

b) levodopa

23) Current enrollment in a structured weight management program or participation in any weight intervention studies in the preceding 90 days

24) Serum ALT and AST greater than 3 x upper limit of normal

25) Hematocrit < 30% or >48% (Since hematocrit values can transiently increase as a result of dehydration, and the subjects in this trial are required to come after an overnight fast, hematocrit levels may be repeated once if the investigators determine that the initial elevated hematocrit was influenced by dehydration)

5.3. Subject Recruitment and Screening

We will use a step process to streamline screening of subjects: telephone screening (step 1), in-person interview and blood tests for blood counts and chemistries, PSA and total and free testosterone, analysis of three 24-hour food recalls, and a Short Physical Performance Battery (step 2). Subjects who respond to our advertisements will undergo telephone screening that conforms to HIPAA guidelines and in which we will verify key inclusion and exclusion criteria by using a standardized questionnaire. Those who conform to the eligibility criteria during the phone screen will be invited to come to the BWH, where they will sign the consent form after a detailed explanation of the study, and undergo medical history, physical examination, blood counts and chemistries, and measurement of PSA. The subjects will also undergo a SPPB. Eligibility will be determined in two screening visits (screening visits SV1 and SV2) based on conformity to inclusion and exclusion criteria using structured interview, questionnaires, physical examination, blood tests, ECG, 24-hour food recalls, and an SPPB test.

5.4. Early Withdrawal of Subjects

Because these trials are based on the principle of “intent-to-treat”, this requires that to the degree possible, all subjects randomized are followed to obtain outcomes data,

regardless of whether they complete their intervention regimens or are noncompliant. This preserves the effect of randomization. Therefore, ideally, a subject would be followed for all outcomes even in the case where he has elected cessation of study intervention.

Therefore the study design explicitly differentiates between cessation of study intervention and withdrawal from study. The subject may elect either at any time. The former may also be elected by the study physician (due for instance to safety or compliance concerns as explained above), but we anticipate that the study physician would invoke the latter quite rarely, if at all.

If a subject decides to cease study intervention, this will be recorded on **Weekly Participant Status Form** and he will no longer receive the intervention to which he has been randomized. Under this scenario study visits would continue as scheduled and the subject would continue to be followed for all endpoints (including adverse events, physical performance testing, etc.) This scenario would also apply if the study physician elects to stop intervention.

If by contrast the subject elects full withdrawal from study, this would be recorded on **Participant Final Disposition Form** and all contact with the subject would cease. If the request for study withdrawal is not communicated in person (i.e. the request is received over the phone), staff will request a final in-person visit with the subject to complete a final safety evaluation prior to cessation of contact with subject. The subject may obviously refuse this request but past experience indicates that subjects are usually willing to be seen for a final disposition visit.

5.5. Restrictions during the course of the study

The participants will be advised that their participation in the study is contingent upon an agreement not to:

- Engage in progressive and heavy weight lifting exercise during the course of the study. The subjects are allowed to continue mild to moderate endurance exercise provided they agree not to change the intensity of the exercise.
- Participate in a structured weight loss program
- Start a nutritional supplement other than that provided by the study staff during the course of the study

6. Study Drug

6.1. Description

The study drug is testosterone enanthate (Delatestryl, ENDO Pharmaceuticals).

6.2. Treatment Regimen

The subjects will receive either placebo or testosterone enanthate 100 mg intramuscularly weekly for six months. All injections will be administered in the GCRU or the study clinic at BWH.

6.3. Drug Storage

Bulk supplies of study medication will be stored in the IDS Pharmacy at controlled room temperature (20-25 Celsius). Study medication will be labeled by the IDS Pharmacy for individual study subjects.

6.4. Subject Randomization and Stratification

Subjects who meet the eligibility criteria will be randomly assigned to one of four treatment groups based on a stratified randomization schedule developed by the biostatistician, using the permuted blocks strategy with randomly varying blocks of size 4 and 8. Stratification will be by diabetes status.

Group A: Placebo injections weekly; 0.8 g/kg/day protein

Group B: Placebo injections weekly; 1.3 g/kg/day protein

Group C: Testosterone enanthate 100 mg intramuscularly weekly; 0.8 g • kg • day protein

Group D: Testosterone enanthate 100 mg intramuscularly weekly; 1.3 g • kg • day protein

6.5. Blinding

The subjects, the research coordinators, and the personnel involved in outcomes assessments and monitoring compliance will all be blinded. Treatment assignment will be known only to the Data Management Team and the Investigational Drug Pharmacy. The testosterone enanthate injections and the sesame oil placebo injections will have identical appearance and consistency. An unblinded physician will be designated to monitor safety results. All participants will receive packaged meals plus a supplement to ensure blinding. The dietary supplements containing either casein and whey mix or carbohydrate and fat mix will have identical appearance.

7. Study Procedures and Visits

7.1. Telephone Screening

Potential subjects who call the trial site in response to advertisements or letters will be asked a set of structured questions to verify key eligibility criteria.

If a potential subject is willing to answer questions, is ≥65 years old and meets the inclusion and exclusion criteria that are assessed during the telephone interview, he will be asked to schedule Visit 1, the first in-person visit.

7.1.1. Visit 1 (V1)

Subjects will first be asked to give written, informed consent.

Visit 1 - Assessments and Procedures

- Screening Consent
- Medical history
- Medications
- Questionnaires: AUA/IPSS
- Height and weight (for BMI)
- Blood pressure
- MMSE
- SPPB
- 24-hour food recall

At V1, The study dietitian will perform a 24-hour food recall and instruct the subjects on how two additional 24-hour recalls will be conducted. For all subjects meeting the eligibility criteria assessed at V1, two additional 24-hour recalls will be completed on randomly assigned days between V1 and V2. The subjects, who meet the eligibility criteria for protein consumption, as well as all other criteria assessed during V1, will be asked to return for V2.

Blood pressure may be repeated at visit 2 if the participant forgot to take antihypertensive medications at visit 1. Blood pressure may also be repeated at visit 2 or if the participant had elevated blood pressure at visit 1 but is interested in participating in the trial and wants to speak with his PCP regarding medication adjustment.

7.1.2. Visit 2 (V2)

The following procedures and questionnaires will be completed:

- Physical examination, including a digital rectal examination (DRE)
- Blood draw for CBC, chemistry panel, PSA, A1C
- ECG
- Rapid assessment of physical activity form (RAPA)
- 24-hour food recall

- Body weight
- Adverse event and concomitant medication will be reviewed

Eligibility will be determined at the completion of V2 and after the results of the tests performed during V2 become available. Subjects who meet ALL the inclusion criteria and NONE of the exclusion criteria will be scheduled for enrollment in the Run-in Period.

Subjects may be asked to return for a repeat PSA, TSH or Hematocrit (rationale elaborated on pages 15 and 16).

7.1.3. The Run-in-period

During the run-in-period, the subjects will be provided a diet containing 0.8g/kg/day protein for a duration of 10 to 16 days. Body weight will be recorded weekly. Adverse event and concomitant medication will be reviewed during this period. Subjects who complete the run-in-period successfully will be eligible for randomization. Subjects may be excluded at the end of the Run-in Period on the basis of the following: (a) failure to return for the baseline study visit, (b) failure to consume at least 75% of the meals provided for reasons of palatability or tolerability, (c) taste aversion to supplements, or (d) any other reason cited by the study investigator or medical team that would interfere with the subject's completion of the 6-month trial. The investigator may exclude subjects even if they meet all the listed eligibility if the investigator determines that participation in the study may be harmful to the participant's health, or if the investigators feel that the subject may not be able to comply with the protocol for any reason.

If a subject meets all eligibility criteria except the 75% compliance with the meals, due to intolerance of or dislike of food items in the study meals, they will be offered a modified meal plan. The modified (or hybrid) meal plan will allow subjects to replace either lunch, dinner or both meals with meals cooked by the subjects themselves. The study dietitian will instruct these subjects on how to prepare their own meals based on their caloric and protein goals. The run-in period of subjects requiring the modified meal plan may be extended by approximately one week. Subjects who are determined to be compliant after the hybrid-run-in phase will be eligible for randomization.

Baseline assessments will be completed for all subjects during or at the end of the run-in period after the eligibility has been determined.

7.1.4. Baseline Visit

After full consent is obtained, the following baseline studies and procedures will be performed:

- Blood tests:
  - Blood counts
  - Blood chemistries, including ALT, AST, bilirubin and alkaline phosphatase, BUN, creatinine, prealbumin, transferrin, and albumin
  - Plasma lipids and apolipoproteins

- Serum PSA
- Two ten ml blood samples drawn ten minutes apart and pooled for the measurement of total and free testosterone, DHT, estradiol, LH, and SHBG.

- ECG
- Anthropometrics. Weight (same scale, minimal clothing) will be recorded.
- Body Composition by a whole body DXA scan
- Muscle performance (maximal voluntary strength, leg power)
- Physical function (6-minute walk, loaded and unloaded stair climbing power and speed, load carrying walk test)

- Questionnaires: SF-36, Psychological Wellbeing Index, DABS, the FACIT-1, and RAPA
- 24-hour urine collection for calcium, total nitrogen and creatinine
- Structured assessment of cardiovascular events

If the subject is completing a second run-in period on the hybrid meal plan, evaluation of meal compliance will take place prior to consenting for the full study.

7.2. Randomization

The subjects will be randomized at the completion of the baseline assessments. The day of randomization will be called day 0.

7.3. Intervention Period

The subjects will be scheduled for weekly injections of study medication at the time of their randomization. Body weight will be recorded weekly. Adverse events and concomitant medications will be reviewed at each visit. Subjects will return to the study clinic each week to receive intramuscular injection of their study medication.

Day 0, Day of Randomization (Visit 6)

- Randomization
- Injection of study medication
- Dietary instructions
- Dispense food and study supplement
- Dispense vitamin and calcium supplement

Week 1 (Visit 7)

- Injection of study medication
- Dispense food
- Dietary instructions
- Dietary checklist

Week 2 (Visit 8)

- Injection of study medication
- Dispense food and study supplement
- Structured evaluation of cardiovascular events
- Vital signs
- 24-hour food recall to verify dietary compliance

Week 3 (Visit 9)

- Injection of study medication
- Dispense food
- Dietary checklist

Week 4 (Visit 10)

- Injection of study medication
- Vital signs
- Dispense food and study supplement
- Dispense vitamin and calcium supplement
- 24-hour food recall for dietary compliance
- Optional RAPA for diet level adjustment

Week 5 (Visit 11)

- Injection of study medication
- Dispense food
- Dietary checklist

Week 6 (Visit 12)

- Injection of study medication
- Physical examination, including a digital prostate examination
- Blood test for CBC, chemistry panel, and hormones
- Urinary symptom assessment with AUA/IPSS
- Dispense food and study supplement
- 24-hour food recall for dietary compliance

Week 7 (Visit 13)

- Injection of study medication
- Dispense food
- Dietary checklist

Week 8 (Visit 14)

- Injection of study medication
- Dispense food and study supplement
- Dispense vitamin and calcium supplement
- 24-hour food recall for dietary compliance

Week 9 (Visit 15)

- Injection of study medication
- Dispense food
- Dietary checklist

Week 10 (Visit 16)

- Injection of study medication
- Dispense food and study supplement
- 24-hour food recall for dietary compliance

Week 11 (Visit 17)

- Injection of study medication
- Dispense food
- Dietary checklist

Week 12 (Visit 18)

- Injection of study medication
- Physical examination, including digital prostate examination
- Blood test for CBC, chemistry panel, PSA, and hormones
- ECG
- Urinary symptom assessment with AUA/IPSS
- Structured evaluation of cardiovascular events and other adverse events
- Dispense food and study supplement
- Dispense vitamin and calcium supplement
- 24-hour food recall for dietary compliance
- DXA
- Muscle performance and physical function assessment
- Questionnaires: SF-36, AUA/IPSS, Psychological Wellbeing Index, DABS, the FACIT-1, and RAPA
- Optional diet adjustment based on RAPA score
- 24-hour urine collection for calcium, total nitrogen and creatinine

Week 13 (Visit 19)

- Injection of study medication
- Dispense food
- Muscle performance and physical function assessment
- Dietary checklist

Week 14 (Visit 20)

- Injection of study medication
- Vital signs
- Dispense food and study supplement
- 24-hour food recall for dietary compliance

Week 15 (Visit 21)

- Injection of study medication
- Dispense food
- Dietary checklist
- Adverse event recording

Week 16 (Visit 22)

- Injection of study medication
- Vital signs
- Dispense food and study supplement
- Dispense vitamin and calcium supplement
- 24-hour food recall for dietary compliance

Week 17 (Visit 23)

- Injection of study medication
- Dispense food
- Dietary checklist

Week 18 (Visit 24)

- Injection of study medication
- Vital signs
- Dispense food and study supplement
- 24-hour food recall for dietary compliance
- Urinary symptom assessment with AUA/IPSS
- Physical examination, including a digital prostate examination
- Blood test for CBC, chemistry panel, and hormones

Week 19 (Visit 25)

- Injection of study medication
- Dispense food
- Dietary checklist

Week 20 (Visit 26)

- Injection of study medication
- Vital signs
- Dispense food and study supplement
- Dispense vitamin and calcium supplement
- 24-hour food recall for dietary compliance

Week 21 (Visit 27)

- Injection of study medication
- Dispense food
- Dietary checklist

Week 22 (Visit 28)

- Injection of study medication
- Vital signs
- Dispense food and study supplement
- 24-hour food recall for dietary compliance

Week 23 (Visit 29)

- Injection of study medication
- Dispense food
- Dietary checklist

Week 24 (Visit 30)

- Injection of study medication
- Physical examination, including a digital prostate examination
- Blood test for CBC, chemistry panel, PSA, and hormones
- ECG
- Structured evaluation of cardiovascular events
- Dispense food and study supplement
- Dispense vitamin and calcium supplement
- 24-hour food recall for dietary compliance
- DXA
- Muscle performance and physical function assessment
- Questionnaires: SF-36, AUA/IPSS, Psychological Wellbeing Index, DABS, the FACIT-1, and RAPA
- Urinary symptom assessment with AUA/IPSS
- 24-hour urine for calcium, total nitrogen, and creatinine

Week 25 (Visit 31)

- Muscle performance and physical function assessment

7.4. Subject Compensation

Subjects will be compensated during the course of the trial, based on the number of visits completed. In addition, parking and meal tickets will be provided for study visits.

8. Dietary Prescription, and Enhancing and Verifying Dietary Compliance

8.1. Standardizing energy and protein content through packaged meals and supplement

Each subject's daily energy requirements will be calculated using the Dietary Reference Intake equation plus an activity factor (Rapid Assessment of Physical Activity; RAPA); this method has been validated in controlled feeding studies (181). Two levels of protein intake will be used: 0.8 g • kg^{-1}• day^{-1} (reference or control group) or 1.3 g • kg^{-1} • day^{-1} (intervention group). The daily energy and protein allowances will be apportioned

between the study meals (pre-packaged or subject-prepared), the protein or energy (placebo) supplements, and a discretionary allowance. We will provide 80% of daily energy allowance through the packaged meals, leaving 20% of energy to be derived from the dietary supplements and discretionary calories. This approach offers flexibility and convenience and increases the likelihood of dietary adherence and maintenance of consistent portion control. Some subjects will be allowed to replace the study-provided meal (lunch and dinner only) with a meal they prepare themselves. Replacements will not be allowed for breakfast or the nutritional supplement. Subjects who are preparing their own lunch or dinner will be taught how to match the daily energy allowance amount as calculated by the Dietary Reference Intake equation plus an activity factor.

Personal Chefs to Go, Inc. will provide meals with only five graded levels of protein and caloric content. Thus, it is likely that the protein and energy content of the packaged meal will not match the estimated protein or energy requirement. The difference between the estimated protein and energy requirement per protocol and the protein and energy content of packaged meal will be made up by a protein and/or carbohydrate supplement. These meals will provide 0.7 g • kg^{-1}• day^{-1} of protein for subjects in the intervention and control groups. Calories in the packaged meals will be apportioned such that 20% of total calories are provided as breakfast, 35% as lunch, and 45% as dinner based on typical distribution of calories across meals in older participants of the Framingham Heart Study. Subjects who are preparing their own lunch or dinner will be taught how to match the total calories and protein content of the packaged meals.

Subjects in the control group will receive all of their daily protein allotment (0.8 g • kg^{-1}• day^{-1}) through packaged meals provided by Personal Chefs To Go, Inc., supplement and discretionary calories (snacks). Subjects in the intervention arm will receive an additional supplement containing 0.5 g • kg^{-1}• day^{-1} of a casein and whey protein mix, bringing their daily protein intake to 1.3 g • kg^{-1}• day^{-1}. To ensure comparability between the two groups, the control group will receive an additional supplement containing 0.5 g • kg^{-1}• day^{-1} of a carbohydrate and fat mix (matched to the protein supplement in taste, texture and caloric content). The supplements will be divided into three portions to be ingested at breakfast, 3-4 PM (with a snack), and at bedtime. Subjects who are preparing their own lunch or dinner will be taught how to match the total calories and protein content of the packaged meals.

To allow for some variety and subject choice, 15% of daily calories will be allocated as discretionary calories and will include such items as fruits and vegetables, coffee and tea with milk and/or sugar, alcoholic beverages, and other foods. The participants will record all discretionary items consumed on the Food Compliance Checklist provided with the packaged meals; Compliance Checklists will be collected every other week at the clinic visit.

The table below illustrates how calories and protein may be distributed in the dietary prescription of a 70-kg person assigned to either the control (0.8 g • kg^{-1}• day^{-1}) or intervention (1.3 g • kg^{-1}• day^{-1}) group and assumed to have daily energy requirement of 2100 Kcal is shown in the table below:

Table 8. Energy and Protein Distribution in a 70-kg Man Assigned to either 0.8 or 1.3 g • kg^{-1}• day^{-1} Groups with estimated daily energy requirement of 2100 kcal

Group assignment	Energy in packaged	Protein in pkg'd	Energy in Supplement	Protein in Supplement	Discret. Calories	Discret. protein	Total energy	Total protein

	meals	meals						
0.8 g/kg/day	1600 kcal	45 g	185 kcal	4	315 kcal	7 g	2100 kcal	56 g
1.3 g/kg/day	1600 kcal	45 g	185 kcal	39 g	315 kcal	7 g	2100 kcal	91 g

Each participant will receive instructions explaining how to apportion, store, and eat the packaged meals and supplements throughout the day. They will also be instructed in the types and amounts of discretionary foods they can eat to add variety. The men assigned to the low and high protein groups will receive identical packaged meals and supplement will be designed to look and taste alike. The importance of dietary compliance will be reinforced every week. The subjects will be provided a seven day supply of packaged food each time. They will pick up their weekly supply of food during their weekly visits to the study clinic or the meals will be shipped to their homes.

Vitamins, minerals and micronutrients

To assure that subjects receive sufficient amounts of vitamins and minerals, they will receive one generic multivitamin tablet, and a generic calcium citrate tablet containing 630 mg of elemental calcium twice daily and 500 IU of vitamin D (the RDA).

Preparation of the Dietary Supplements

The dietary supplements will be prepared by the manufacturers. For the intervention group received the 0.5 $g \cdot kg^{-1} \cdot day^{-1}$ protein supplement, the supplement itself will be prepared by mixing Milk Protein Concentrate-80 (MPC-80) with Whey Protein Concentrate-80 (WPC-80). The 80:20 casein to whey blend of MPC-80 will be diluted to a 50:50 casein:whey blend (CWB 50:50) using an appropriate amount of WPC-80. The final CWB 50:50 will be unflavored to provide the greatest flexibility to the subjects for mixing and consuming the supplements at home. However, the protein supplement will be sweetened to match the taste of the carbohydrate (glucose-based) supplement. Quality control over the blend of casein to whey as well as the total protein concentration per unit of powder will be carried out for each batch at the manufacturing plant and results of the quality control analysis will be provided to the investigators with the shipment of each new batch.

The protein and carbohydrate supplements will be delivered to the study dieticians in tubs for the purpose of individual packaging for study subjects. Supplements will be packaged in individual portions with the name, ID number and day and date for consumption. Color coding will be used to separate morning, afternoon and evening supplement doses.

8.3. Compliance with Dietary Prescription

We have incorporated several approaches to enhance dietary compliance, including the provision of packaged meals and portion control, the use of compliance checklists to assess compliance on an every two-week basis, and the use of this information in real time to reinforce dietary instructions every other week.

In this study, subjects are provided pre-packaged frozen meals and protein/placebo supplements. Some subjects will be allowed to replace the study-provided meal (lunch and dinner only) with a meal they prepare themselves. Replacements will not be allowed for breakfast or the nutritional supplement. Subjects who are preparing their own lunch or dinner will be taught how to match the daily calories and protein content as calculated by the Dietary Reference Intake equation plus an activity factor. They will also be given recommendations to not eat out more than one meal per week.

For subjects with lactose intolerance, the study dietician devises a specific meal plan to minimize dairy in the meals. However; as dairy is part of many food products and not completely avoidable, some subjects could experience gastrointestinal symptoms while on study meals. In

order to improve compliance in these subjects we will prescribe lactase supplements to symptomatic subjects. The lactase supplement would be packaged by our IDS Pharmacy following the same procedures that are in place for the dispensing of multivitamin and calcium/vitamin D supplements for this study.

Compliance with dietary prescription will be assessed by using alternating Compliance Checklists and 24-hour food recalls during alternating weeks. The subjects will return their Compliance Checklist at every other weekly visit. Subjects who are preparing their own lunch or dinner will be asked to keep detailed food records for 2 week days and 2 weekend days during alternating weeks. These subjects will return their Compliance Checklist at every other weekly visit. These subjects will continue to receive 24-hour food recalls every other week.

Due to the large respondent burden associated with returning empty meal containers and incompletely-consumed meals, especially in older subjects with mobility problems, we will not require the return of such items. Instead, we will use a Dietary Compliance Checklist, a method that has been used successfully in previous studies by these investigators. The Dietary Compliance Checklist is a natural choice for a feeding study because of its simplicity, objectivity, and precision. The checklist, included with each meal, will list each item that is included in the meal package and the respondent will simply check off all foods eaten. For those foods that are not completely eaten, the subject will indicate what portion was consumed (e.g., one-quarter or one-half). Subjects will return their Compliance Checklists every other weekly visit.

The participants will be instructed on how to fill out Dietary Compliance Checklist and will be given handouts with examples of completed food records. At every other weekly visit, the study nutritionist will review the Dietary Compliance Checklist and inquire about difficulties associated with compliance, including the amount of food provided, palatability, problems in filling the Compliance Checklist, and factors affecting the ability to follow the prescribed diet or supplements. The compliance will be calculated as the percent of daily energy and protein eaten, using the NDS-R, and averaged across the treatment period to obtain an average compliance score for each subject. The biweekly 24-hour food recalls will be used as a cross-check on the compliance estimates obtained from the Compliance Checklists. The compliance estimates will be used a covariate in secondary analyses. These methods are established approaches to monitor compliance in dietary intervention studies and based on more than 10 years experience of Dr. Apovian.

8.4. Exercise and Activity

All participants will be asked to abstain from resistance and heavy endurance exercise. Those involved in weight lifting exercises will be enrolled only if they agree to discontinue weight lifting at least twelve weeks before the start of the study. Those involved in mild to moderate aerobic exercises on a regular basis may continue to do so provided the intensity of exercise is maintained constant throughout the study period.

9. Statistical Plan

9.1. Analytical Methods and Sample Size Estimations

Statistical Analyses. Primary analyses will follow the “intention-to-treat” (ITT) principle; i.e., individuals will be analyzed according to their assigned treatment group whether or not they remain on the study treatment. Every attempt will be made to follow and continue to evaluate all enrolled subjects. The ITT approach avoids bias if individuals who drop out of the two arms have different prognoses. For safety

outcomes, however, all individuals exposed to testosterone, whether or not it was their assigned treatment, will be considered. No assumptions will be made as to the consistency of the rate of change in outcomes with time. A secondary, 'per-protocol,' analysis will also be conducted for each outcome with noncompliant subjects excluded. All analyses will be adjusted for randomization and stratification factors. The subjects will be randomized in equal parts to one of two protein regimens (0.8 g/kg/d vs. 1.3 g/kg/d), and within each of these to testosterone or placebo, so that four subcohorts (coded A-D in the table below) of equal size may be compared to one another to assess the marginal and joint influences of testosterone and enhanced protein intake. The primary outcome is lean body mass, measured by DXA, and the primary focus of the statistical analysis estimation and inference surrounding the effects of 1.3 g diet and testosterone supplementation on lean body mass above and beyond those attributable to 0.8 g protein and placebo, respectively. Leg press strength, chest press strength, leg power, walking speed, stair climbing power, load carrying speed, self-reported physical function, fatigue, well being and affectivity balance will be considered as secondary outcomes. Based on extensive experience, we assume an overall rate of attrition of no more than 20% of subjects during the six-month trial period (see below).

Descriptive and Exploratory Analyses. Prior to formal modeling, exploratory methods will be employed to assess data quality, preliminary evidence of associations and to detect the presence of outlying values and potential influence points. Means, standard deviations, medians, and ranges will be computed for continuous variables, and sample probability mass functions computed for categorical factors. Histograms, scatter plots, and box plots will be used to assess skew and examine assumptions (such as normality) underlying statistical models. Baseline characteristics for the four randomized subcohorts will be compared using ANOVA for continuous variables and Fisher's exact tests for categorical variables. Where appropriate, log or power transformations of outcome variables will be employed to enhance conformity with assumptions (e.g. normality in outcomes and/or linearity in associations between outcomes and covariates). Exploratory graphical assessment of the strength of association between covariates and outcome variables will be obtained via scatterplot smoothing using Generalized Additive Models. A preliminary assessment of the quantity and pattern of missing data will also be performed.

Compliance Analysis. Compliance will be evaluated in two areas: dietary compliance and the number of injections. Dietary compliance will be assessed by means of alternating Dietary Compliance Checklist and 24-hour food recalls as described in another section. The percent of injections received at the weekly clinic visits will be calculated. The primary intent to treat analysis will include all subjects; we will also perform secondary analyses using compliers.

Hypothesis Testing. The primary analysis of lean body mass will be performed in two parts. Our primary analytical strategy is to use mixed-effects regression analysis to assess 3 and 6 month outcomes simultaneously with baseline total body lean mass considered as a covariate, as is appropriate for between-group comparisons of randomized subcohorts, and 6-month differences between arms will be estimated via a treatment contrast and corresponding 95% confidence interval. We will also use a two-way ANOVA to compare change from baseline to 6-month across the four randomized

subcohorts. We expect general agreement between estimates arising from Parts I and II, but owing to its use of all data, that from mixed effects regression analysis will be considered the principal estimate of treatment effect. Though the primary aim of the analysis will be to estimate treatment effects at 6-months, a linear trend in change with time will not be assumed, and the potential for differential mean trajectories in treatment effects over the entire duration of follow-up will be assessed via stratification and the use of statistical interaction terms. Unless otherwise noted, all comparisons will assume an allowed type-I error probability of 0.05.

Hypothesis 1 is concerned with the effect of 1.3 g • kg^{-1} • day^{-1} protein administration on body composition, strength and function as compared to the effects of RDA (0.8 g • kg^{-1} • day^{-1}). The effect of enhanced protein intake will be tested using a mixed effects regression model on all subjects, comparing those with enhanced protein to RDA controlling for administration (or lack of administration) of testosterone as well as stratification factors and baseline outcome measures (i.e. an ANCOVA scheme). Modification of the protein effect by testosterone administration will be investigated by the use of statistical interaction terms, which will be considered significant if achieving the 0.10 threshold.

Hypothesis 2 is concerned with the differential effect of testosterone administration in the enhanced protein administration arms vs the RDA arms. Again a mixed effects regression analysis will be performed, restricted to subjects receiving testosterone. Comparison of baseline to follow-up change in outcomes will then be performed between the protein subcohorts (1.3 g / k/day vs 0.8 g/k/day), controlling for stratification factors. Analyses of secondary outcomes will proceed according to the same program. As secondary, exploratory analyses, we are also interested in knowing whether changes in lower extremity lean mass (measured by DEXA) correlate with changes in leg press strength and physical function. For these variables, we will generate the Pearson correlation coefficients. Appropriate scatter plots will be used to assess assumptions of linearity and homoscedasticity. If necessary, Spearman correlations will be computed to confirm the main results. We will also fit regression models on the change in leg press strength with terms for group, baseline protein intake, baseline testosterone levels, and the group by baseline testosterone interaction to assess if treatment works to a greater or lesser extent in men with low testosterone or low protein intake.

We will examine whether changes in muscle strength correlate with changes in the measures of physical function (6-minute walking speed and time, stair climbing power), and self-reported physical function. If testosterone treatment does improve muscle strength, and also improves physical function and self-reported physical function, it would be of interest to know whether changes in muscle strength correlate with changes in self-reported and objective measures of physical function.

Missing data. Primary analyses will employ mixed-effects regression in order to utilize all available data under the ITT framework. No assumptions will be made concerning data lost to attrition (e.g. crude imputation techniques such as last-observation-carried-forward will not be employed). No technique can perfectly assess the precise influence of missing data. We will therefore employ sensitivity analyses to assess the potential

influence of missingness on conclusions, as is advocated by numerous authors; moreover, outcomes data collection will continue regardless of compliance with randomly assigned regimens, thus minimizing the influence of missingness under ITT. For instance we will compare baseline outcome and covariate values for all subjects with missing outcomes vis-a-vis those with complete data. A lack of systematic differences is consistent with but does not prove the robustness of conclusions to bias from the missing data process. The conclusions of these sensitivity analyses will be incorporated in published reports of findings from the proposed study.

Multiple Comparisons: We recognize that a number of hypotheses will be tested, which will increase the likelihood of committing a type 1 error. We are aware of many different approaches that can be used to adjust for multiple comparisons. A two -sided, 5% level of significance will be used for each **pre-specified** hypothesis and magnitude of effect size will be carefully evaluated for clinical significance. For exploratory hypotheses, we will adjust the p value by using the Boneferroni's correction. It is still possible that we will find statistical significance in some instances when the differences are of little clinical significance. In such cases, **the clinical importance of findings** – whether the magnitude of treatment effect meets or exceeds the minimal clinically important difference in the outcome - will be highlighted in the interpretation of results.

b. Sample Size Estimate (Approved by the DSMB in its June 2016 meeting)

We estimate that **92 men** will be needed to test the primary hypothesis. We base this estimate on the following considerations:

- Two-sided, type I error rate of 0.05.
- We assume that the groups will be balanced at baseline due to successful randomization.
- The primary analysis will use an intent-to-treat strategy in which all will be analyzed as randomized.

- Baseline calculations below apply to Part A of the primary analysis, which is restricted to comparisons at 6 months controlling for baseline outcomes measurements using a linear regression model with estimates of both active treatment effects simultaneously. Part B (mixed effects modeling) will achieve greater power than is claimed below.
- Additive intervention effects. The potential for synergistic influence of interventions will be considered in exploratory analyses.
- We assume that no more than 15% of subjects in four groups will drop out before end of study. The dropout rate in our previous testosterone dose response study in older men which had rigorous dietary requirements was 12%.

Primary comparisons. This study will be powered to detect clinically meaningful differences in within-subject changes in lean body mass attributable to randomization to testosterone therapy or 1.3 g protein diet above and beyond those attributable to placebo and 0.8 g protein diet, respectively. Interaction between the interventions will be addressed in exploratory analyses, but will not be the primary focus of these computations.

The expected pattern of gain in lean body mass among the four randomization groups (A, B, C, D) is depicted in the table below. We anticipate that the group of subjects receiving placebo treatment and the 0.8 g protein diet will experience some marginal gain in lean body mass, denoted μ. Subjects receiving the elevated protein diet and / or testosterone will exhibit greater gains, with the apparent effects of testosterone and 1.3 g diet exhibiting an additive relation. The anticipated mean ± SD gain in lean body mass attributable to testosterone therapy in this design (denoted in the table as the difference between cohorts A and C, or B and D) is expected to be approximately 2.8 kg with standard deviation no greater than 4.2 kg - yielding an effect size of at least 0.67 – while the gain attributable to 1.3 g diet is anticipated to be approximately 1.3 kg with SD about 2.3 kg, yielding an effect size of approximately 0.65. These estimates are reasonable and conservative because in our testosterone dose response study, older men receiving the same dose of testosterone and 1.2 g protein• kg • day gained 4.3 kg (SD 2.3 kg, Preliminary Data) lean body mass.

If the hypothesized differences hold, a sample size of 19 participants in each of the four treatment groups (total sample size = 76 men) would provide 80% power to detect these effects, while group-specific evaluable sample sizes of 26 and 29 would provide approximately 85% and 90% power to detect these effects, respectively. To insure 80% power for primary comparisons we therefore propose an enrollment sample of 76 / 0.85, i.e. 92 participants or 23 per cell.

Although based on previous studies, we have hypothesized that there will no significant differences in change in lean body mass in older individuals receiving 0.8 g protein or 1.3 g protein, the proposed sample size will be able to detect a difference of 1.3 kg in

the change in lean body mass between these two groups (SD 2.3 kg), which we posit is clinically meaningful.

Secondary comparisons. The anticipated analytic sample size of 92 subjects provides sufficient power to detect clinically meaningful changes in most of the secondary outcomes with 80% power. We anticipate, for instance, that the change in leg press strength in testosterone plus 0.8 g protein group will be 15 kg (SD 24 kg) and in testosterone plus 1.3 g protein group will be 24 kg (SD 30 Kg). This assumption is conservative based on previous testosterone trial which revealed an increase of 28 kg in leg press strength (SD 22 kg). We anticipate therefore that the true effect size of the

testosterone effect on leg press strength will be approximately 0.56, detectable with 80% power under the proposed design. We assert that a 15 kg increase in leg press strength is clinically significant based on the fact that Fiatarone et al demonstrated that a 10 kg increase in leg press strength was associated with significant improvements in physical function, as assessed by stair climbing power, walking speed, and time for sit-to-stand transition. The proposed sample size will allow us to detect a difference of 10 points in physical function domain of SF-36 HRQOL instrument in testosterone and placebo-treated men and a difference of 15 points between testosterone plus 1.3 g protein and placebo plus 0.8 g protein with >80% power. These differences are clinically meaningful as Ware et al have deemed a change of 8 point in the physical function domain of SF-36 to be clinically meaningful. The proposed sample size will allow us to detect this effect size with 80% power.

Table 1. Anticipated mean change in lean body mass according to protein diet and assignment to testosterone or placebo therapy. The baseline expected increase μ present in the placebo + 0.8 g reference cell (upper left) will be augmented in the other subcohorts by the additive effect of additional protein intake and/or Testosterone therapy.

Protein Regimen	Placebo	T
0.8 g / d	**A**: μ	**C**: μ + 2.8 kg
1.3 g / d	**B**: μ + 1.5 kg	**D**: μ + 4.3 kg

9.2. Adverse Events

We will compare proportions of men experiencing adverse events in each treatment group, with particular attention to areas that are plausibly associated with testosterone, including erythrocytosis, urinary tract symptoms, and prostate-related events or to protein intake such as kidney stones or hypercalciurea.

9.3. Interim monitoring

Once in progress, the study will be monitored by the Data and Safety Monitoring Board (DSMB), an independent group that will periodically review the results in order to assess safety. No interim efficacy assessment is planned. The DSMB will receive semi-annual

reports and will meet annually, The DSMB will decide if an early stopping rule would be used to recommend termination of the study.

10. Safety and Adverse Events

10.1. Definitions

Definitions are per the January 2007 Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events, Office on Human Research Protection (OHRP) Guidance. http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm. The requirements and processes for reporting adverse events are described in the corresponding National Institute on Aging (NIA) Guidelines.

10.1.1. Adverse Event

An *adverse event (AE)* is any untoward or unfavorable medical occurrence in a human study participant, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the participant's involvement in the research, whether or not considered related to the subject's participation in the research.

10.1.2. Serious Adverse Event

A *serious adverse event* (SAE*)* is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- results in congenital anomalies or birth defects
- an important medical event*

Important medical events* are those that may not be immediately life threatening, but are clearly of major clinical significance.

10.1.3. Unanticipated Problem

An Unanticipated Problem is any incident, experience, or outcome that meets all of the following criteria:

- unexpected (in terms of nature, severity, or frequency) given the research procedures that are described in the IRB-approved research protocol and informed consent document;
- related or possibly related to participation in the research; possible related means that there is a reasonable possibility that the incident, experience or outcome may have been caused by the procedures involved in the research.

- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) related to the research than was previously known or recognized.

10.1.4. Adverse Event Reporting Period

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up.

10.1.5. Preexisting Condition

A preexisting condition is one that is present at the time of signing the consent form for the main study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

10.1.6. General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

10.1.7. Post-study Adverse Event

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study DCC of any death or adverse event occurring during the year after a subject has completed treatment.

10.1.8. Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management

10.1.9. Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization should be documented and reported as a serious adverse event. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event and reported as a severe adverse event if hospitalization is required. Neither the condition, hospitalization, nor surgery are reported as an adverse event if the hospitalization was for diagnostic or elective surgical procedures for a preexisting condition.

10.2. Recording of Adverse Events

At each contact with the subject, the investigator or study staff will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document. All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs during the year after completion of treatment will similarly be recorded and reported.

10.3. Reporting of Serious Adverse Events

10.3.1. Study Sponsor Notification by Investigator

The study team will report serious adverse events to the DSMB, within 48 hours of first knowledge of the event. Serious Adverse Event (SAE) form must be completed by the investigator and faxed to the study sponsor within 24 hours. The investigator will keep a copy of this SAE form on file at the study site. A summary report of the adverse events will be submitted to the IRB every six months along with the DSMB report.

10.4. Termination Criteria

The following **Termination Criteria** will be used to guide discontinuation of study medication to minimize the risks of drug-related adverse events. However, all subjects will be followed in conformity with intent-to-treat analytical strategy.

- Increase in serum creatinine >2.5 mg/dL, only if confirmed by a repeat test
- 24-hour urinary calcium excretion rate > 400 mg; if the 24-hour collection is deemed erroneous by the study clinician, the test will be repeated.

If either of these two criteria is met, the nutrition intervention will be discontinued.

In addition, in accordance with the Endocrine Society and ISSAM guidelines for testosterone therapy, we will use the following termination criteria related to testosterone therapy:

- PSA increment of >1.4 ng/ml above baseline, if confirmed by a repeat test
- Detection of prostate or breast cancer
- Hematocrit >54%, if confirmed by a repeat test
- Myocardial infarction

- Stroke

If any of these criteria is met, the study medication (testosterone or placebo) will be discontinued.

We recognize that other unexpected adverse events can occur. The study physician may stop study medication for other adverse events if in his/her opinion, continuation of study medication would pose an unacceptable risk to the subject's health. We have instituted a comprehensive plan for early detection of adverse events. The Data Safety Monitoring Board is empowered to discontinue treatment in one or more subjects, or halt the study, should the occurrence of adverse events so warrant.

10.5. Unblinding

Treatment assignment will be blinded to all but a single designated "unblinded" physician or the research pharmacist. Although testosterone treatment might increase the risk of certain diseases, such as prostate cancer, lower urinary tract symptoms due to benign prostatic hyperplasia, or erythrocytosis, the blind will not be broken even if a subject develops one of these conditions during the study. Instead, the following approach will be taken.

a. If a subject is diagnosed with prostate cancer during the study, treatment will be discontinued, whether the treatment is testosterone or placebo.
b. If the subject's score on the International Prostate Symptoms Score increases by ≥5 points during treatment, suggesting worsening of lower urinary tract symptoms, the subject will be evaluated for medications that affect urine flow rates and for prostatitis, and treatment with an alpha blocker and urologic evaluation will be considered.
c. If a subject develops a hematocrit >54%, we will recheck hematocrit. If confirmed, study medication will be stopped.

11. Monitoring Subject Safety

11.1. Potential Risks to Subjects

Several conditions to which elderly men are particularly prone may be worsened by testosterone.

The potential risks of the study include the risks of blood drawing, exercise testing, high protein intake in excess of RDA, testosterone administration, DXA scan, and the administration of questionnaires.

Risks of Blood Drawing. The risks of blood drawing include pain, bleeding, and/or a bruise at the site of needle insertion. Serious complications such as blood clots or infection are very rare when proper precautions are taken.

Potential Risks of Protein Intake in Excess of RDA. There are no long term, adequately powered studies to determine the adverse health consequences of

eucaloric, high protein diets in older individuals. Several safety concerns have been voiced, including the risk of renal dysfunction, hypercalciurea, and osteoporosis. Additionally, people often perceive high protein diets as being "unhealthy" – containing "too much fat or animal products". A meta-analysis has confirmed that for every 25-g increase in dietary protein, urinary calcium increases by 0.8 mmol. There is concern that increased calcium exertion during ingestion of high protein diets may reflect bone demineralization and increase the risk of kidney stones. Increased amounts of dietary protein also might be expected to increase endogenous acid production and bone may be called upon to buffer the additional acid load, resulting in mobilization of bone calcium, and a decline in bone mineral density (BMD). A few cross-sectional studies have reported a positive association between dietary protein intake and hip fracture incidence. However, bone balance studies have not found an increase in bone calcium resorption. Instead, Kerstetter et al found that additional dietary protein promotes intestinal calcium absorption and reduces the fraction of urinary calcium of bone origin. Also at odds with the bone hypothesis are a large number of epidemiological studies showing that a high-protein diet is associated with high, not low BMD. The net effect of dietary protein on bone mineral status depends on dietary availability of calcium; a diet rich in protein, dairy, and calcium attenuates urinary calcium and bone loss relative to a high carbohydrate diet.

Additional concerns have been voiced about potential adverse effects of high protein diet on renal function; Brenner had hypothesized that high protein intakes by diabetic individuals create hyperfiltration and glomerular hypertension resulting in renal damage; however, this hypothesis has not found strong support. There is no evidence that high protein intakes up to 2 g $\cdot kg^{-1} \cdot d^{-1}$ increase the risk of renal impairment in individuals with normal renal function. However, in individuals with pre-existing impairment of renal function, high protein intake greater than 45% of energy intake may accelerate the decline in renal function. The amount of protein being investigated in this study (0.8 and 1.3 g • kg^{-1} • day^{-1}) is lower than the levels that have been associated with renal impairment. Also, we will exclude individuals with estimated GFR <60 ml/min. We will monitor serum creatinine and 24-hour calcium excretion during the course of the study.

Potential Adverse Effects of Testosterone. The 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, weight gain and change in body habitus. Serious side effects such as hepatotoxicity and hepatic neoplasms, anecdotally identified with oral administration of high dose, long-term administration of 17-alpha-alkylated derivatives of testosterone in athletes, have not been observed in men when testosterone is administered parenterally or transdermally. In addition, there is concern and uncertainty about the long term risks of prostate and cardiovascular disorders in older men treated with testosterone. A recent testosterone trial in older men with mobility limitations reported a higher frequency of cardiovascular events in men assigned to the testosterone arm than in those assigned to placebo arm of the study (see below).

Testosterone supplementation increases red cell mass and this is reflected in increased hemoglobin and hematocrit. The increment in most men with low testosterone levels is

modest, and is clinically not significant. However, in some older men, particularly in those who are smokers, the increase in hemoglobin might be greater. Therefore, we will monitor hemoglobin levels during the course of the study, and increments in hemoglobin levels above 54% will warrant discontinuation of testosterone treatment, referral to a specialist for evaluation and treatment including therapeutic phlebotomy if indicated by the occurrence of neuro-occlusive symptoms. In older men, testosterone administration has been associated with significantly greater increments in hematocrit than that observed in younger men. We will monitor hematocrit, and discontinue treatment, should hematocrit rise above 54% or should neuro-occlusive symptoms occur at any hematocrit.

Testosterone administration can induce or exacerbate pre-existing sleep apnea. However, the frequency of this adverse event is low in young as well as older men. In our previous studies of carefully selected, older men, similar to those that will be enrolled in the proposed studies, we did not observe this complication in any participant. Snyder et al also did not observe a significant increase in the frequency of sleep apnea in older men treated with a scrotal patch for three years in comparison to those who received the placebo patches.

Testosterone can also cause transient sodium and water retention; this could potentially cause edema or exacerbate congestive heart failure in men with pre-existing cardiac dysfunction. We will exclude men with CHF with Class III or IV symptoms, or those with myocardial infarction in the preceding 6 months.

Although the data from experimental animals provides unequivocal evidence that testosterone regulates territoriality, scent marking behavior, and aggression in male mammals, particularly at the time of mate selection, the data in humans are highly equivocal. There are anecdotal reports of “roid rage” among men abusing large doses of androgenic steroids. However, systemic investigation has not revealed a significant increase in aggressive behaviors in carefully screened men given testosterone enanthate in doses of up to 600-mg weekly. We cannot exclude the possibility that still higher doses of androgenic steroids when administered to men with pre-existing personality disorders or psychopathology might induce aggression. In this study, we will use a replacement dose of testosterone gel that is expected to produce average serum testosterone concentrations in the mid to high normal range; we are not aware of any reports of “roid rage” reactions at these testosterone concentrations in either men or women.

Testosterone and Prostate Cancer Risk. There is agreement that testosterone does not cause prostate cancer. Also, epidemiological studies have failed to demonstrate a consistent, dose-related, correlation between prostate cancer risk and sex hormone levels. However, there are a number of areas of uncertainty that are discussed below.

i. Prostate cancer is a common, androgen–dependent tumor, and androgen administration may promote tumor growth. Testosterone administration is absolutely contraindicated in men with history of prostate cancer.

ii. Many older men have microscopic foci of cancer in their prostates. We do not know whether testosterone administration will make these subclinical foci of cancer grow and become clinically overt.

iii. In addition, older men with prostate cancer may have low testosterone levels. Morgentaler et al reported a high prevalence of biopsy-detectable prostate cancer in men with low total or free testosterone levels despite normal PSA levels and results of digital rectal examination. However, this study did not have a control group, and we do not know whether sextant biopsies of age-matched controls with normal testosterone levels would yield a similarly high incidence of biopsy-detectable cancer.

iv. Serum PSA levels are lower in testosterone–deficient men and are restored to normal following testosterone replacement. However, serum PSA levels do not increase progressively in healthy hypogonadal men with replacement doses of testosterone. In four placebo–controlled trials of testosterone administration in older men, the change in serum PSA levels over three–years was not significantly different between placebo–and testosterone–treated men. The increase in PSA levels during testosterone replacement might trigger evaluation and biopsy in some patients.

v. More intensive PSA screening and follow-up of men receiving testosterone replacement might lead to increased number of prostate biopsies and detection of subclinical prostate cancers that would have otherwise remained undetected.

vi. Serum PSA levels tend to fluctuate when measured repeatedly in the same individual over time. Therefore, when serum PSA levels in androgen deficient men on testosterone replacement therapy show a change from a previously measured value, the investigator will need to decide whether the change warrants detailed evaluation of the patient for prostate cancer, or whether it is simply due to test–to–test variability in PSA measurement.

Testosterone Effects on the Risk of Heart Disease. The long-term consequences of testosterone supplementation on the risk of heart disease remain unknown. The clinical trials data on the effects of testosterone supplementation on cardiovascular risk are limited by small size of many trials, relatively short intervention periods, and poor quality of adverse event recording and adjudication. Meta-analyses of randomized trials have failed to reveal statistically significant difference between the testosterone and placebo arms. In the TOM Trial, a randomized, placebo-controlled testosterone trial in older men with mobility limitation, men assigned to the testosterone arm of the study experienced greater number of adverse events, particularly cardiovascular events than those assigned to the placebo arm of the study. Therefore, in the proposed study, we will use a structured instrument for prospective recording of cardiovascular events. We will use a lower dose of testosterone enanthate than we had planned originally. We will exclude men with myocardial infarction or stroke in the preceding six months. The study duration is not long enough to permit meaningful evaluation of atherosclerosis progression or cardiovascular event rates.

While supraphysiological doses of non-aromatizable, 17-alpha-alkylated, orally administered androgens undoubtedly decrease plasma HDL-cholesterol levels, physiologic testosterone replacement administered either parenterally or transdermally has been associated with only a modest decrease in plasma HDL-cholesterol. Thus, previous studies in which supraphysiological doses of 17-alpha-methyl testosterone have been administered to women have demonstrated a decrease in plasma HDL-cholesterol.

Cross-sectional studies of middle-aged men find a direct, rather than an inverse, relationship between serum testosterone levels and plasma HDL-cholesterol concentrations, and an inverse correlation between testosterone levels and visceral fat volume in older men have demonstrated no significant change in plasma HDL-cholesterol levels during long-term testosterone administration. Similarly, epidemiological studies indicate an inverse relationship between circulating androgen concentrations and carotid intima-media thickness; thus men and women in the highest quartile of androgen concentrations have the least intima media thickness.

Spontaneous and experimentally induced androgen deficiency is associated with increased fat mass, and testosterone replacement decreases fat mass in older men with low testosterone levels. Testosterone supplementation of middle-aged men with truncal obesity is associated with a reduction in visceral fat volume, serum glucose concentration, blood pressure, and an improvement in insulin sensitivity. Surgical castration in rats impairs insulin sensitivity; physiologic testosterone replacement reverses this metabolic derangement. However, high doses of testosterone impair insulin sensitivity in castrated rats. These data suggest that both high and low concentrations of testosterone might have deleterious effects on insulin sensitivity.

Whether variation of testosterone within the normal range is associated with risk of coronary artery disease remains controversial. Of the 30 cross-sectional studies reviewed by Alexandersen, 18 reported lower testosterone levels in men with coronary heart disease, 11 found similar testosterone levels in controls and men with coronary artery disease and 1 found higher levels of DHEAS. Prospective studies have failed to reveal an association of total testosterone levels and onset of coronary disease.

Testosterone increases coronary blood flow by direct effects on blood vessel wall. In an intervention study, testosterone undecanoate given orally improved angina pectoris in men with coronary heart disease. Testosterone infusion acutely improves coronary blood flow in a canine model and in men with coronary artery disease.

Testosterone levels are lower in patients with type 2 diabetes mellitus compared with controls. Low total and free testosterone levels are associated with a higher risk of the developing type 2 diabetes. Free testosterone levels are negatively correlated with glucose, insulin, and C-peptide levels independent of body mass index. Some of these studies measured free testosterone by a tracer analog method, which is not independent of SHBG effects. Lower testosterone levels in men are associated with higher levels of dense LDL particles and prothrombotic factors.

In a mouse model of atherosclerosis that is LDL-receptor deficient surgical castration accelerates, and testosterone administration retards the progression of atherosclerosis.

The magnitude of testosterone effect on atherosclerosis progression is similar to that observed with estrogen administration. Favorable effects of testosterone on atherosclerosis in this mouse model are antagonized by concomitant administration of an aromatase inhibitor, suggesting that testosterone effects are possibly mediated through its conversion to estrogen in the vessel wall. Testosterone effects in retarding atheroslcerosis progression were independent of plasma lipids.

Potential Risks of DXA Scanning. The radiation exposure during a DXA scan is minimal (less than 25 mrems, less than that from a chest X ray). While we do not know whether any dose of radiation is completely safe, this amount of radiation to which the subjects will be exposed during the course of the study is well within the limits considered "safe" by federal and state regulations.

Potential Risks of Physical Function Testing. There is a small risk of incurring injury during assessment of muscle performance and physical function. This risk will be minimized by properly instructing the participants, and by performing these tests under direct supervision of an experienced exercise technician. Gordon et al have reported on the safety of maximal strength testing in 6,653 males and females aged 20-69 years. Of this sample, 1112 subjects were over 50. Only one of the 6663 subjects experienced a clinically significant, non-fatal or fatal cardiovascular event in association with strength testing. The authors complemented these data with two additional data sets: 1) 1819 maximal strength assessments performed at the Cooper Clinic (Dallas, TX) and 2) approximately 4500 different subjects who completed approximately 20,000 maximal strength tests at the University of Florida's Center for Exercise Science. These subjects were aged 18-93 years. Shaw and colleagues studied maximal strength testing in 83 elderly persons with a mean age of 66 ±6 years using the same 1RM methodology proposed for this study. Strength in five resistance training exercises was determined in these subjects. Eighty-one of the subjects completed the testing without injury, 48 complained of muscle soreness after testing, and 2 subjects (2.4%) sustained an injury. Bassey using the same equipment for testing leg power proposed in this study, successfully tested 26 frail elderly male and female residents of a chronic care hospital, These subjects averaged 88.5 ± 6 years for the men and 86.5± 6 years for the women and had multiple pathologies. In addition to the leg power testing, all subjects completed a battery of four physical function tests including a 4-step stair climb, a 6 meter walk, and repetitive rising from a chair. No injuries or adverse effects from the testing procedures was reported. Fiatarone et al measured maximal voluntary strength in nonageranians without problems. Taken together, these data strongly suggest that maximal strength and power, and physical function testing can be performed safely in older men.

Our group has extensive experience in the application of the muscle function assessments proposed for this study including studies in older healthy men, older men with COPD, and older men with low testosterone (Please, see Drs. Bhasin and Storer's biosketches). We have conducted these tests in young and older men and women without incident for over 20 years. We attribute this in part to the general safety of the tests as indicated above as well as through application of careful and systematically applied testing procedures. We will conduct strength testing with Keiser pneumatic

equipment that allows as little as one pound increments in resistance, thus maintaining the ability to make appropriately small increments in resistance as required in older, frail individuals. This equipment has been used extensively in aging research and shown to be safe when used with elderly persons. The risks of strength and power testing include muscle soreness and injury. These risks will be minimized by following careful procedures (standardized protocols) for warm-ups, progressive increase in workload, and careful supervision of the subjects during their testing by trained and experienced technicians. We will employ careful instruction, demonstration and provide practice sessions so that subjects may become familiar with the procedures.

Potential Risks of Questionnaires. The instruments used for the assessment of self-reported physical function, disability, affect, sense of well being and fatigue ask about personal details that the subjects might find embarrassing. Although we encourage subjects to answer all the questions, they have the option of not answering the questions that they might find embarrassing. All the questionnaires are coded and confidentiality is maintained at all times.

Difficulties in Obtaining Informed Consent when there is Uncertainty about the Potential Risks. The purpose of the informed consent process is to provide a full disclosure of the potential risks so that the participants can perform a meaningful risk:benefits assessment. However, we also recognize that communicating this information effectively to research subjects is a complex and difficult job. In view of the growing media coverage of this issue, the research subjects may have misconceptions about the potential benefits. Also, the absence of definitive data makes it difficult to provide quantitative estimates of the risk; hence, the subjects may not comprehend the magnitude of the risk to their health.

In an attempt to provide as full a disclosure as possible, we have built in a multi-step informational process that starts with subject's first telephone call, and continues with the subject's visit to the CTSI/GCRU when further details of the study are provided and the subject's questions are answered before the subject signs the consent form. We recognize that the process of obtaining informed consent is a continuous process that does not end when the subject signs the consent form. Thus the process of providing information about the risks and benefits will continue throughout the course of the project.

11.2. Procedures for Protecting Against Potential Risks

All subjects will be monitored by frequent physical examinations, blood counts and chemistries. We will also monitor PSA, digital prostate examination, hemoglobin and hematocrit, 24-hour urinary calcium excretion, and creatinine levels. In addition, the Research Coordinator will be in frequent contact with the subjects. We will ascertain adverse events reported by the subjects at baseline, at 6 weeks, 3 months and 6 months.

The risks of blood drawing will be minimized in that only experienced practitioners (licensed physicians and clinical research nurses) will perform phlebotomy.

The risk of injury from participating in exercise testing will be minimized by several safety measures. The subjects will be instructed in proper technique and the risks minimized by careful supervision. Patients will be screened with exercise stress testing to rule out cardiac ischemia and arrhythmias. This test will be done in the presence of an experienced physician. The likelihood of musculoskeletal injury from exercise testing will be minimized by providing a warm-up period proceeding each exercise session including warm-up stretching exercises. Further, an experienced exercise trainer will oversee all exercise testing sessions.

11.3. Data and Safety Monitoring Board

An external DSMB will be established by the NIA to monitor all aspects of the study. The Board will consist of experts in geriatrics, biostatistics, clinical trials, and nutrition. The DSMB members will not be affiliated with the study and will be appointed by the NIA Director. The Board will meet every six months to review subjects' safety, study progress and data integrity and completeness. After each meeting, the DSMB will provide the NIA Director with its recommendations, and the Director will decide whether to accept them. The study team will submit the DSMB report to the Partners IRB.

12. Data Management

The Boston University Data Coordinating Center will provide data management and statistical programming under the supervision of the investigators and biostatistician. This will be accomplished by the successful completion of the following tasks:

Design Randomization through a Web Application: An automated randomization application will be available. Upon completion of Inclusion/Exclusion form, a new subject ID will be generated and the randomization table, stratified by diabetes status will be queried for study arm designation.

Design database for subject enrollment and tracking: A database system will be designed to track subjects, provide documentation of eligibility and agreement to participate or the reason for refusal, and, whether the subject signed the informed consent document. The database will provide automatic alerts to the study coordinators about due dates for follow-up visits. The database will reside on a secure, password protected SQL server; only connections from users authenticated from the domain controller will be accepted, providing a secure environment. The database will be automatically backed up on a nightly basis.

Design data collection forms: The Data Management team will prepare data collection forms creating a standardized data collection infrastructure. Forms will be designed to ensure that all data fields are unambiguous, items are easily read, response categories are in standardized units, and the instructions are clearly worded.

Data Capture and Quality Control: The completed forms will be entered into a web-based database system. A unique ID will be used to identify individual forms; subject names will not be recorded. After verification, the data will be saved to the database (Microsoft SQL server). If an error is detected, the form will not be committed to the

database until resolved. After forms have been verified, computer algorithms will be written in SAS to check internal inconsistencies.

Dietary data will be entered by a research nutritionist into the **Nutrition Data System (NDS)** of the University of Minnesota. Similarly, the 24-hour food recalls collected during the study will be reviewed and the subject debriefed prior to entry of the data by a research nutritionist into the Nutrition Data System (NDS) of the University of Minnesota.

Create analytic datasets: All dietary data will be cleaned by an experienced nutrition data manager. A statistical programmer will be responsible for cleaning other data, as well as creating analytic data sets and carrying out data analyses. Documentation will include the creation of standardized protocols for coding and entering dietary and other data, creation of a data dictionary that includes all original and composite or created variables. The statistical programmer, under the supervision of the project Statistician, will write the SAS code for all analyses for DSMB reports, progress reports, and manuscripts.

Data Security: The Data Management Team has in place standard procedures for database backup to prevent data loss, systems validation, and performance monitoring. User access will be controlled by assignment of usernames and passwords. The DCC systems meet the regulatory requirements for electronic records described in Code of Federal Regulations (CFR) 21 Part 11, and the applicable regulatory guidelines.

Maintaining Blinding. The Data Management Team will collaborate with the Investigational Drug Service and the unblinded physician to protect the blinding of treatment assignments. Electronic access to information that could indirectly or directly lead to unblinding will be limited and such information will be stored in password-protected files.

12.1.1. Maintaining Anonymity of Submitted Medical Records

Clinical site personnel will de-identify all medical records before sending them to the DCC by obliterating any Protected Health Information (PHI). Upon receipt, DCC personnel will review the records to ensure that no PHI is visible.

12.1.2. Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act (HIPAA) of 1996.

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