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Design of a randomized trial to determine the optimum protein intake to preserve lean body mass and to optimize response to a promyogenic anabolic agent in older men with physical functional limitation

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Abstract

The dietary protein allowance for older men to maintain lean body mass and muscle strength and to accrue optimal anabolic responses to promyogenic stimuli is poorly characterized. The OPTIMEN trial was designed to assess in older men with moderate physical dysfunction and insufficient habitual protein intake (< recommended dietary allowance, RDA, 0.8 $g \cdot kg^{-1} \cdot d^{-1}$) the efficacy of consuming diets containing 163% RDA (1.3 $g \cdot kg^{-1} \cdot d^{-1}$) for protein, compared to RDA,

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to increase lean mass, muscle performance, and physical function. A second aim was to determine whether increasing protein intake to 1.3 versus 0.8 $g \cdot kg^{-1} \cdot d^{-1}$ would augment anabolic responses to a promyogenic agent, testosterone.

For this randomized, double-blind, placebo-controlled six-month intervention trial, 92 men, 65 years or older, with Short Physical Performance Battery scores 3–10, and habitual protein intakes < RDA, were assigned to one of four groups: 100% RDA plus placebo intramuscular injections weekly; 100% RDA plus weekly intramuscular injections of 100 mg testosterone enanthate; 163% RDA plus placebo injections; or 163% RDA plus testosterone injections. All participants received portion-controlled packaged meals and group-specific dietary supplements containing either mixtures of casein and whey or carbohydrate, with identical appearance. The primary outcome was change in lean body mass assessed using dual energy X-ray absorptiometry. Secondary outcomes included maximal voluntary strength and power in leg press and chest press exercises, 6-minute walking distance, stair climbing power, and self-reported physical function.

Results of the OPTIMEN trial have important implications for dietary protein guidance and policy, and efficacy of promyogenic drugs.

1. Introduction

The recommended dietary allowance (RDA) for protein has been set at 0.8 $g \cdot kg^{-1} \cdot day^{-1}$; this allowance is intended to meet the needs of the entire adult population – men and women, young and old [1]. This value represents the minimum amount of protein required to avoid loss of lean body mass in most individuals. However, the protein requirements in older adults remain poorly validated and have engendered vigorous debate [2–7]. Many experts advocate dietary protein intakes substantially above the current RDA to maintain muscle anabolism in older individuals; these scientists cite studies which suggest that protein intakes above the RDA for older adults have beneficial anabolic effects on the skeletal muscle [2,3,7–14]. Other experts support the current protein RDA [4,5]. The efficacy of protein intakes in excess of the RDA in improving muscle mass, muscle performance and physical function has not been demonstrated in older individuals and is an issue with health policy implications.

A US Department of Agriculture (USDA) survey revealed that about a third of older Americans do not ingest the RDA for protein [15]; other surveys have confirmed these findings [16]. Low protein intake has been implicated as a contributor to the multifactorial pathophysiology of sarcopenia, the aging-associated loss of muscle mass and function [2,7,9,11–12]. However, we do not know whether increasing protein intake in older adults, whose protein intake is below the RDA, increases muscle mass, muscle performance and physical function. Therefore, the first aim of the Optimizing Protein Intake in Older Men with Mobility Limitation (OPTIMEN) Trial was to determine whether administration of 1.3 $g \cdot kg^{-1} \cdot day^{-1}$ of protein results in greater improvements than the RDA (0.8 $g \cdot kg^{-1} \cdot day^{-1}$) in lean body mass, maximal voluntary muscle strength and power, and physical function in older men whose protein intake is less than the RDA and in whom energy intake has been standardized at 30 $kcal \cdot kg^{-1} \cdot day^{-1}$. We selected 0.8 $g \cdot kg^{-1} \cdot day^{-1}$ and 1.3 $g \cdot kg^{-1} \cdot day^{-1}$ as the two levels of protein intake for comparison. The 0.8 $g \cdot kg^{-1} \cdot day^{-1}$ is the RDA and, therefore,

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the reference level of protein intake. Many nutrition experts recommend that an intake of 1.3 $g \cdot kg^{-1} \cdot day^{-1}$, or about 15–20% of total caloric intake, is necessary to optimize health outcomes in older individuals [2–3,7–9]. Although a few experts have recommended protein intakes even higher than 1.3 $g \cdot kg^{-1} \cdot day^{-1}$, protein intakes in excess of 1.3 $g \cdot kg^{-1} \cdot day^{-1}$ may be difficult to achieve in free living older adults because a vast majority of older individuals eat < 1.3 $g \cdot kg^{-1} \cdot day^{-1}$. Our protein intake target of 1.3 $g \cdot kg^{-1} \cdot day^{-1}$ is therefore more likely to be achievable in the average older person, and also likely to be an effective dose for demonstrating a difference in muscle mass accretion when compared to the RDA.

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 to optimize adaptations to exercise training [17–19]. However, studies have failed to demonstrate improvements in lean body mass or physical function in older individuals fed higher amounts of protein in excess of the RDA. This failure could be a function of the recruitment of subjects with varying levels of physical function and sarcopenia. The protein requirements for achieving optimal anabolic response to administration of promyogenic function promoting therapies are unknown. Therefore, the second aim was to test the hypothesis that the RDA of 0.8 $g \cdot kg^{-1} \cdot day^{-1}$ protein intake is insufficient to attain optimal anabolic response to administration of a promyogenic agent, such as testosterone. We hypothesized that in older adults whose daily protein intake is less than the RDA, increasing dietary protein intake to 1.3 $g \cdot kg^{-1} \cdot day^{-1}$ without changing the total daily energy intake, will augment anabolic response to testosterone and will be associated with greater gains in lean body mass and maximal voluntary strength than the RDA.

2. Study design

The OPTIMEN trial was a randomized, placebo-controlled, parallel group, double blind, clinical trial in community dwelling, older men, 65 years or older, who have moderate degree of physical dysfunction. The study had a 2 × 2 factorial design to enable us to investigate the effects of dietary protein intake and testosterone separately and together. The study protocol was approved by the institutional review boards for Humans Subjects Research at Boston University Medical Center and the Brigham and Women's Hospital.

2.1. Participant selection – eligibility criteria

The eligibility criteria were designed to select older men, who have a moderate degree of physical dysfunction, whose protein intake is less than the 0.83 $g \cdot kg^{-1} \cdot day^{-1}$, who were able to provide informed consent and participate in study assessments, and who did not have conditions that would render them susceptible to increased risk of adverse effects of testosterone or of protein intake above the RDA (Table 1).

We recruited men whose daily protein intake was < 0.83 $g \cdot kg^{-1} \cdot day^{-1}$ based on an average of 3 days of 24-hour dietary recalls. The cutoff value of 0.83 $g \cdot kg^{-1} \cdot day^{-1}$ was selected as the exact 97.5 percentile for protein requirements from the meta-analysis of nitrogen balance studies by Rand et al. [20]. For inclusion, we required an intake of < 0.83 $g \cdot kg^{-1} \cdot day^{-1}$ of protein on 2 out of 3 dietary recalls or from the average of 3 dietary recalls. We included

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men with moderate degree of physical dysfunction as indicated by a Short Physical Performance Battery (SPPB) score of 3 to 10 because men with minimal or no physical limitation, being close to the ceiling of performance in this test, are unlikely to show improvements in their performance in this test, while those with SPPB score < 3 may be too limited to show improvement – a case of too little, too late [21–22].

We excluded men who had conditions, which might be potentially exacerbated by testosterone [23], such as prostate cancer, untreated severe sleep apnea, untreated heart failure, recent major adverse cardiovascular event, or erythrocytosis. We excluded men who were unwilling or unable to provide an informed consent, such as those with dementia, and those who were unable to participate in study assessments, such as the men with limiting musculoskeletal conditions or stroke with residual neurological deficit. We also excluded men who had symptoms of severe lower urinary tract obstruction, as defined by a score of > 19 on the International Prostate Symptom Score (IPSS) questionnaire.

To ensure that the participants included in the study comply with the dietary prescription, we excluded participants, who, during the two-week run-in period, had taken in < 75% of the meals and/or less 75% of the supplement.

2.2. Subject recruitment and screening

We used 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, and the SPPB (step 2). Additionally, 24-hour food recalls on three separate days, including one weekend day, were analyzed by a registered dietitian to ascertain average daily energy and protein intake.

Subjects who responded to our advertisements or to the mailings underwent telephone screening that conformed to HIPAA guidelines and in which we verified key inclusion and exclusion criteria by using a standardized questionnaire. Those who met the eligibility criteria during the telephone screen were invited to come to the research unit, where they signed the informed consent form after a detailed explanation of the study, and underwent medical history, physical examination, blood counts and chemistries, and measurement of PSA. The subjects also underwent a SPPB.

2.3. The run-in-period to determine compliance with the prescribed dietary regimen and acceptability of the prescribed custom diet

During the run-in-period, the subjects were provided a custom diet containing 0.8 g/kg/day protein for a duration of 10 to 16 days. Body weight was recorded weekly. Adverse events and concomitant medications were reviewed every two weeks during this period. Subjects who completed the run-in-period successfully were eligible for randomization. Subjects were excluded during the run-in period on the basis of the following: (a) failure to return for the scheduled study visits, (b) failure to consume at least 75% of the provided meals and/or supplements for reasons of palatability or tolerability, (c) an expressed 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. Baseline assessments

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were completed for all subjects at the end of the run-in period after eligibility had been determined.

2.4. Participant allocation

The participants, who meet the eligibility criteria were randomly assigned to one of four intervention groups using a concealed randomization schedule developed by the biostatistician, using the permuted blocks strategy with randomly varying blocks of size 4 and 8, as follows: Group A: Placebo injections weekly plus 0.8 g/kg/day protein; Group B: Placebo injections weekly plus 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 plus 1.3 g·kg·day protein.

2.5. Blinding

The participants, the research staff, and the personnel involved in outcomes assessments were all blinded. Treatment assignment was known only to the Data Management Team and the Investigational Drug Pharmacy. An unblinded physician was designated to monitor safety results. All participants received packaged meals plus a supplement to ensure blinding. The dietary supplements containing either casein and whey mix or carbohydrate had identical appearance.

2.6. Study medication

The participants received weekly intramuscular injections of either testosterone enanthate 100 mg or an equal volume of placebo. The injections were administered by study staff in the research unit.

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

Each subject's daily energy requirement was calculated using the Dietary Reference Intake equation plus an activity factor; this method has been validated in controlled feeding studies. Two levels of protein intake were used: 0.8 $g \cdot kg^{-1} \cdot day^{-1}$ (reference or control group) or 1.3 $g \cdot kg^{-1} \cdot day^{-1}$ (intervention group). The daily energy and protein allowances were apportioned between the pre-packaged study meals, the protein or energy (placebo) supplements, and a discretionary allowance. Eighty percent of daily energy allowance was provided through the packaged meals, leaving 20% of energy to be derived from the dietary supplements and discretionary calories. This approach offered flexibility and convenience and was expected to increase dietary adherence and maintenance of consistent portion control.

A commercial vendor (Personal Chef to Go, Mechanicsville, VA) provided meals with five graded levels of protein and caloric content to enhance feasibility of the nutrition intervention. We anticipated that the protein and energy content of the packaged meals may not match the estimated protein or energy requirement. The difference between the estimated protein and energy requirement and the protein and energy content of the packaged meals was made up by a protein and/or carbohydrate supplement. These meals provided 0.7 $g \cdot kg^{-1} \cdot day^{-1}$ of protein for subjects in the intervention and control groups. Calories in the packaged meals were apportioned such that 20% of total calories were provided as breakfast,

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40% as lunch, and 40% as dinner, thereby enabling the lunch and dinner meals to be interchangeable to allow the subjects more choice in meal selection.

The participants in the control group received all of their daily protein allotment (0.8 g·kg^{-1}·day^{-1}) through packaged meals provided by the commercial vendor, supplement and discretionary calories (snacks). The participants in the intervention arm received 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}. The discretionary calories provided 0.1 g/kg/day protein to add up to either 0.8 g·kg^{-1}·day^{-1} for the control or 1.3 g·kg^{-1}·day^{-1} for the intervention group. To ensure comparability between the two groups, the control group received an additional supplement containing 0.5 g·kg^{-1}·day^{-1} of a carbohydrate powder. The supplements were divided into three portions to be ingested as a mid-morning snack, 3–4 PM (with a snack), and at bedtime.

To allow for some variety and subject choice, 15% of daily calories was allocated as discretionary calories and included such items as fruits and vegetables, coffee and tea with milk and/or sugar, alcoholic beverages, and other foods. The participants recorded all discretionary items consumed on the Food Compliance Checklist provided with the packaged meals. Compliance Checklists were collected every other week during the research center visit.

Each participant received instructions explaining how to apportion, store, and eat the packaged meals and supplements throughout the day. The participants were also instructed in the types and amounts of discretionary foods they could eat to add variety. The men assigned to the low and high protein groups received identical packaged meals and supplements were designed to look and taste alike. The importance of dietary compliance was reinforced every week. The subjects were provided a seven-day supply of packaged food each time. The weekly supply of thermally regulated food was either picked up by the participants during their weekly visits to the study clinic or was delivered by an overnight courier to their homes on a weekly basis during a specified window of time when the participant was at home and could immediately refrigerate the received food supply.

To ensure that they receive sufficient amounts of vitamins and minerals, the participants received one multivitamin tablet, and a calcium citrate tablet containing 630 mg of elemental calcium and 500 IU of vitamin D twice daily.

2.8. Preparation of the dietary supplements

The dietary supplements were prepared by the manufacturer (Dairy Research Institute®, Bariatrix Nutrition Corp., and Abbott Laboratories). The participants in the intervention group received the 0.5 g·kg^{-1}·day^{-1} protein supplement. The protein supplement was prepared by mixing Milk Protein Concentrate-80 (MPC-80) with Whey Protein Concentrate-80 (WPC-80). The 80:20 casein to whey blend was diluted using an appropriate amount of WPC-80 to achieve an average of 50:50 casein:whey blend (CWB). The final CWB was unflavored to provide the greatest flexibility to the subjects for mixing and consuming the supplements at home. However, the protein supplement was sweetened to match the taste of the carbohydrate (glucose-based) supplement. Quality control over the

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blend of casein to whey as well as the total protein concentration per unit of powder was carried out for each batch by Rtech Laboratories and results of the quality control analysis were provided to the investigators with the shipment of each new batch.

The supplements were packaged in individual portions for each participant. Color coding was used to separate morning, afternoon and evening supplements.

2.9. Compliance with dietary prescription

We incorporated several approaches to enhance compliance with the dietary regimen, including the provision of packaged meals and portion control, the use of compliance checklists to assess compliance every two weeks, and reinforcement of dietary instructions 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 did not require the return of such items. Instead, we used a Dietary Compliance Checklist, a method that had been used successfully in previous studies by these investigators [24]. The checklist included with each meal lists all items that were included in the meal package; the respondent simply checked off all foods eaten. For those foods that were not completely eaten, the subject indicated what portion was consumed (e.g., one-quarter or one-half). Subjects return their Compliance Checklists during every other weekly visit.

At every other weekly visit, the study nutritionist reviewed the Dietary Compliance Checklist and inquired 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 was calculated as the percent eaten for of the following items: all meals, protein foods, supplements, protein foods plus supplements, all meals and supplements, using the Nutrition Data Systems-Research software (University of Minnesota) (NDS-R), and averaged across the treatment period to obtain an average compliance score for each subject. The biweekly 24-hour food recalls were used as a cross-check on the compliance estimates obtained from the Compliance Checklists.

2.10. Hybrid feeding plan for participants who found the custom diet unpalatable

After about a third of the participants had been enrolled, a review of those who met all other eligibility criteria and had in-range SPPB score but who withdrew prior to randomization revealed that one third of these subjects cited palatability of the prescribed diet as the reason for refusal to participate further in the trial. After approval of the trial's Data and Safety Monitoring Board and the IRB, we modified the study protocol to include the option of a hybrid diet to achieve the dual goals of maintaining stringent control over the protein and energy intake during the trial and of allowing greater choice in the selection of foods in the custom designed diet.

Accordingly, the participants, who did not wish to participate due to inability to enjoy the meals, were given the option of substituting the entrée in the meal provided by the commercial vendor with a home cooked entrée which had the same protein and energy intake as that included in their dietary prescription. The study dietitians instructed the

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participant on how to estimate the macronutrient content of lunches or dinners so that the participant ate the amount of protein and calorie content prescribed. The dietitians used exchange lists for meal planning based on the American Dietetic Association recommendations [25]. Instead of the Compliance Checklist used by the participants, who followed the meal plan offered by the commercial vendor, the participants on the hybrid meal plan completed food logs of the meals taken instead of what was delivered by commercial vendor. These instructions were provided during an initial in-person session with the research dietitian that lasted between 1 and 2 h. Subjects were encouraged to replace as few meals as necessary in order to maintain the study diet.

2.11. Exercise and activity

All participants were asked to abstain from resistance and heavy endurance exercise during the study. Those involved in weight lifting exercises were enrolled only if they agreed 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 were allowed to continue to do so provided the intensity of exercise was maintained constant throughout the study period.

2.12. Study outcomes

The primary outcome of the trial was change in lean body mass, measured by dual energy X-ray absorptiometry (DXA) [26–27], 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 (Table 2). At a population level, sarcopenia defined in terms of lean body mass is predictive of disability, fracture risk, mortality, and other adverse health outcomes. DXA scans were all read by the same personnel at the Bone Unit of Brigham and Women’s Hospital.

We recognized 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 included measures of muscle performance and physical function as secondary outcomes. Maximal voluntary strength measured by the 1-repetition maximum method in the leg press and the chest press exercises were included as secondary outcomes [26–28]. These exercises were chosen because they involve the large muscle groups of the lower and upper extremities. Measures of physical function included 6-minute walking distance and speed; stair-climbing power without and with carrying a load of 20% body mass; and 50-meter timed walk while carrying a 20% body mass load. Assessments of muscle performance and physical function were performed by trained and experienced exercise physiologists in the Function Assessment Core of the Boston Claude D. Pepper Older Americans Independence Center, using standardized methods that have been published [26–28].

We characterized self-reported physical function using the physical function domain of the Medical Outcomes Study Short Form-36 (SF36) [29]. Sense of wellbeing was assessed by Psychological Well Being Index, and fatigue by FACIT-1 Fatigue scale [30]. Positive and negative affect was assessed by Derogatis Affectivity Balance Scale (DABS) [31].

The schedule of study assessments is shown in Table 3.

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2.13. Safety measures

In accordance with the recommendations of the Endocrine Society's Expert Panel for Testosterone Therapy of Androgen Deficiency Syndrome in Men [23], we monitored the following to assure subject safety: hemoglobin and hematocrit; serum PSA levels, periodic digital rectal examinations of the prostate, and American Urological Association/ International Prostate Symptom Score (AUA/IPSS) score; and plasma lipids (Table 4).

There is no evidence that testosterone enanthate, when administered by intramuscular injection at the proposed dose, affects liver enzymes [23]. 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 $g \cdot kg^{-1} \cdot day^{-1}$are associated with adverse health effects, we monitored 24-hour urinary calcium excretion, blood urea nitrogen (BUN) and creatinine, and blood chemistry panel, including albumin, pre-albumin and transferrin as markers of protein status. This study was neither sufficiently long in duration nor was adequately powered to determine the effects on bone mineral density and fracture risk.

2.14. Statistical plan

Primary analyses will follow the "intention-to-treat" (ITT) principle; i.e., individuals will be analyzed according to their assigned intervention group regardless of their compliance or whether or not they remain on the study intervention. For safety outcomes, however, all individuals exposed to testosterone, whether or not it was their assigned treatment, will be considered. A secondary, 'per-protocol,' sensitivity analysis will also be conducted for each outcome with nonadherent subjects excluded.

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 and stair climbing power (both with and without a load carry), self-reported physical function, fatigue, wellbeing and affectivity balance are considered secondary outcomes.

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. Where appropriate, log or power transformations of outcome variables will be employed to enhance conformity with assumptions.

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. Adherent participants will be defined as those with > 90% compliance with the dietary prescription and who took > 90% of their prescribed injections.

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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; 6-month differences between arms will be estimated via a treatment contrast and corresponding 95% confidence interval. All comparisons will assume a type I error probability of 0.05. Modification of the protein effect by testosterone administration will be investigated by the use of statistical interaction terms, which will generally be considered statistically significant if achieving the 0.10 threshold. As we anticipate that the effects of testosterone and protein supplementation may not have additive effects on certain outcomes, the clinical significance of potential interactions will also be assessed.

Analyses of secondary outcomes will proceed according to the same program. As additional secondary analyses, we are also interested in knowing whether changes in lean body mass are related to change in protein intake and change in circulating testosterone levels, and whether the relationship between the change in on-treatment circulating testosterone levels and change in lean body mass is influenced by the level of protein intake (1.3 g·kg·day vs 0.8 g·kg·day).

A two -sided, 5% level of significance will be used for each prespecified hypothesis and no adjustment will be made for multiple comparisons for pre-specified hypotheses. The magnitude of effect size will be carefully evaluated for clinical significance.

2.15. Sample size estimate

We estimated that 92 men will be needed to test the primary hypothesis. We based this estimate on the following considerations: two-sided, type I error rate of 0.05; an intent-to-treat analytical strategy; no > 15% of subjects in four groups dropping out before end of study; and Pearson correlation between baseline and follow-up lean mass measures at least 0.5, consistent with prior studies [26–27]. We hypothesized that subjects receiving the elevated protein diet and testosterone will exhibit greater gains than those receiving placebo injections and 0.83 g/kg protein, with the apparent effects of testosterone and 1.3 g protein diet exhibiting an additive behavior. The anticipated mean ± SD gain in lean body mass attributable to testosterone therapy in this design is expected to be approximately 2.8 kg with standard deviation no > 4.2 kg - yielding an effect size of at least 0.67 – while the gain attributable to 1.5 g diet is anticipated to be approximately 1.5 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·kg·day gained 4.3 kg (SD 2.3 kg) lean body mass [19]. If the hypothesized differences hold, an evaluable sample size of 19 participants in each of the four treatment groups (total evaluable 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.

The anticipated analytic sample size of 92 subjects also 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

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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) [26]. 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 [32].

3. Discussion

Protein requirements for older individuals have been the subject of contentious debate for some time. The RDA estimate has been derived largely from short term studies of two to three week duration, using nitrogen balance studies [1,4–6], which have been criticized on multiple other grounds [4–6]: inclusion of a small number of individuals, inclusion of no or very few older adults, 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 [9]. 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, while others have equally fervently supported the adequacy of the current RDA [4–6,9]. Thus, the issue being addressed in this investigation has enormous public health and policy implications.

We incorporated several strategies - packaged meals, portion control, frequent contact and reinforcements, and frequent checks of dietary compliance in real time by using compliance checklists - which have been shown to enhance compliance with the dietary prescription in previously successful trials, such as the Dietary Approaches to Stop Hypertension (DASH) [32], Diabetes Prevention Program (DPP) [33], and many obesity trials [34]. The inclusion of a hybrid dietary accommodates the needs of our ethnically diverse study population, some of whom find the custom packaged diet unpalatable, and should further increase compliance with the dietary prescription.

The study incorporated all the attributes of good clinical trial design such as randomization, stratification, and blinding, an appropriate sample size based on consideration of effect size and power, attentive safety monitoring, and a multidisciplinary team of experts from several relevant disciplines. The 2 × 2 factorial design should enable us to determine the effects of dietary protein and the pharmacologic anabolic therapy separately and together.

Instead of relying on nitrogen balance studies or biochemical measures of protein turnover, we included lean body mass, which is an outstanding integrated measure of whole body anabolism that can be measured with high level of precision and accuracy even in older adults. In addition to measures of lean body mass, we also included measures of muscle performance and physical function.

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The proposed investigation is novel and innovative in several ways. It investigated the effects of two levels of protein intake on lean body mass, muscle performance and physical function rather than relying on biochemical markers such as nitrogen balance and muscle protein kinetics. Unlike previous studies which have included individuals with varying protein intakes – higher and lower than the RDA - we recruited individuals with protein intake less than the RDA, who have objectively verified physical dysfunction and who are arguably the most likely to benefit from higher protein intake. With a study duration of 6-months and a sample size of 92, this would be one of the longest and the largest studies of this type in older adults. Most other studies have been conducted in the setting of resistance exercise training; our study will determine the effects of varying protein intake in the setting of a pharmacological anabolic intervention. The trial's findings will be of interest to the development of a large number of pharmacological function promoting therapies that are in development, such as the myostatin anatgonists, selective androgen receptor modulators, growth hormone secretagogs, and IGF mimetics. The results of this trial have important implications for nutrition policy. Since older adults undergo age associated muscle loss, it is crucial to determine the optimal protein intake to maintain muscle mass and function and to achieve maximal benefit from anabolic stimulation as a treatment for sarcopenia in the growing population of older adults.

Acknowledgements

Data and Safety Monitoring Board

The National Institute on Aging appointed a Data and Safety Monitoring Board to oversee the trial's progress and participant safety throughout the duration of the trial. The DSMB met through conference calls twice a year to review study's progress and the safety data.

DSMB Chair: Dr. Richard Grimm served as the DSMB chair from the trial's inception until June 2015, when Dr. Connie Bales assumed this position until the end of the trial. Other members of the DSMB included Drs. Peter Peduzzi (2009 to 2014); Anne Kenny; Philip Miller (2015–2017); and Dennis Villareal.

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References

[1]. The National Academies, The Institute of Medicine, Dietary Reference Intakes for Energy, Carbohydrates, Fiber, fat, Protein, and Amino Acids (Macronutrients), The National Academies Press, Washington, DC, 2002/2005.

[2]. Campbell WW, Evans WJ, Protein requirements of elderly people, Eur. J. Clin. Nutr 50 (Suppl. 1) (1996) S180–S183. [PubMed: 8641258]

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[3]. Gaffney-Stomberg E, Insogna KL, Rodriguez NR, Kerstetter JE, Increasing dietary protein requirements in elderly people for optimal muscle and bone health, J. Am. Geriatr. Soc 57 (2009) 1073–1079. [PubMed: 19460090]

[4]. Millward DJ, Sufficient protein for our elders? Am. J. Clin. Nutr 88 (2008) 1187–1188. [PubMed: 18996850]

[5]. Millward DJ, Fereday A, Gibson N, Pacy PJ, Aging, protein requirements, and protein turnover, Am. J. Clin. Nutr 66 (1997) 774–786. [PubMed: 9322550]

[6]. Gersovitz M, Motil K, Munro HN, Scrimshaw NS, Young VR, Human protein requirements: assessment of the adequacy of the current recommended dietary allowance for dietary protein in elderly men and women, Am. J. Clin. Nutr 35 (1982) 6–14. [PubMed: 7064878]

[7]. Wolfe RR, Miller SL, Miller KB, Optimal protein intake in the elderly, Clin. Nutr 27 (2008) 675–684. [PubMed: 18819733]

[8]. Campbell B, Kreider RB, Ziegenfuss T, La Bounty P, Roberts M, Burke D, Landis J, Lopez H, Antonio J, International Society of Sports Nutrition position stand: protein and exercise, J. Int. Soc. Sports Nutr 4 (2007) 8–14. [PubMed: 17908291]

[9]. Campbell WW, Trappe TA, Wolfe RR, Evans WJ, The recommended dietary allowance for protein may not be adequate for older people to maintain skeletal muscle, J. Gerontol. A Biol. Sci. Med. Sci 56 (2001) M373–M380. [PubMed: 11382798]

[10]. Volpi E, Nazemi R, Fujita S, Muscle tissue changes with aging, Curr. Opin. Clin. Nutr. Metab. Care 7 (2004) 405–410. [PubMed: 15192443]

[11]. Houston DK, Nicklas BJ, Ding J, Harris TB, Tylavsky FA, Newman AB, Lee JS, Sahyoun NR, Visser M, Kritchevsky SB, Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study, Am. J. Clin. Nutr 87 (2008) 150–155. [PubMed: 18175749]

[12]. Bales CW, Ritchie CS, Sarcopenia, weight loss, and nutritional frailty in the elderly, Annu. Rev. Nutr 22 (2002) 309–323. [PubMed: 12055348]

[13]. American College of Sports Medicine, American Dietetic Association, Dietitians of Canada, Joint Position Statement: nutrition and athletic performance, Med. Sci. Sports Exerc 32 (2000) 2130–2145. [PubMed: 11128862]

[14]. Paddon-Jones D, Short KR, Campbell WW, Volpi E, Wolfe RR, Role of dietary protein in the sarcopenia of aging, Am. J. Clin. Nutr 87 (2008) 1562S–1566S. [PubMed: 18469288]

[15]. Department of Agriculture, Nutrient Intakes: Survey in 48 States, Year 1977–78, Nationwide Consumption Survey 1977–78, Washington, DC, 1984 (Report No 1–2).

[16]. United States Department of Agriculture, Agricultural Research Service, Tippett Katherine S., Cypel Yasmin S. (Eds.), Design and Operation: The Continuing Survey of Food Intakes by Individuals and the Diet and Health Knowledge Survey, 1994–96, 1997 Nationwide Food Surveys Report No. 96–1, 197.

[17]. Lemon PW, Tarnopolsky MA, MacDougall JD, Atkinson SA, Protein requirements and muscle mass/strength changes during intensive training in novice bodybuilders, J. Appl. Physiol 73 (1992) 767–775. [PubMed: 1400008]

[18]. Tarnopolsky MA, Atkinson SA, MacDougall JD, Chesley A, Phillips S, Schwarcz HP, Evaluation of protein requirements for trained strength athletes, J. Appl. Physiol 73 (1992) 1986–1995. [PubMed: 1474076]

[19]. Meredith CN, Zackin MJ, Frontera WR, Evans WJ, Dietary protein requirements and body protein metabolism in endurance-trained men, J. Appl. Physiol 66 (1989) 2850–2856. [PubMed: 2745350]

[20]. Rand WM, Pellett PL, Young VR, Meta-analysis of nitrogen balance studies for estimating protein requirements in healthy adults, Am. J. Clin. Nutr 77 (1) (1 2003) 109–127. [PubMed: 12499330]

[21]. Guralnik JM, Ferrucci L, Simonsick EM, Salive ME, Wallace RB, Lower-extremity function in persons over the age of 70 years as a predictor of subsequent disability, N. Engl. J. Med 332 (1995) 556–561. [PubMed: 7838189]

[22]. Guralnik JM, Ferrucci L, Pieper CF, Leveille SG, Markides KS, Ostir GV, Studenski S, Berkman LF, Wallace RB, Lower extremity function and subsequent disability: consistency across studies,

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predictive models, and value of gait speed alone compared with the short physical performance battery, J. Gerontol. A Biol. Sci. Med. Sci 55 (2000) M221–M231. [PubMed: 10811152]

[23]. Bhasin S, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, Swerdloff RS, Montori VM, Task Force, Endocrine Society. Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline, J. Clin. Endocrinol. Metab 95 (6) (2010) 2536–2559. [PubMed: 20525905]

[24]. Apovian CM, Bigornia S, Cullum-Dugan D, Schoonmaker C, Inoue J, Phipps J, Gokce N, Istfan N, Meyers A, Lenders CM, Commercial meal replacements and intensive lifestyle in overweight and obese urban low income adolescents – a 12 week safety pilot project. ICAN Infant, Child Adolesc. Nutr 1 (2009) 37–44. [PubMed: 22347517]

[25]. Wheeler ML, Nutrient database for the 2003 exchange lists for meal planning, J. Am. Diet. Assoc 103 (2003) 894–920. [PubMed: 12830032]

[26]. Bhasin S, Woodhouse L, Casaburi R, Singh AB, Mac RP, Lee M, Yarasheski KE, Sinha-Hikim I, Dzekov C, Dzekov J, Magliano L, Storer TW, Older men are as responsive as young men to the anabolic effects of graded doses of testosterone on the skeletal muscle, J. Clin. Endocrinol. Metab 90 (2005) 678–688. [PubMed: 15562020]

[27]. Travison TG, Basaria S, Storer TW, Jette AM, Miciek R, Farwell WR, Choong K, Lakshman K, Mazer NA, Coviello AD, Knapp PE, Ulloor J, Zhang A, Brooks B, Nguyen AH, Eder R, LeBrasseur N, Elmi A, Appleman E, Hede-Brierley L, Bhasin G, Bhatia A, Lazzari A, Davis S, Ni P, Collins L, Bhasin S, Clinical meaningfulness of the changes in muscle performance and physical function associated with testosterone administration in older men with mobility limitation, J. Gerontol. A Biol. Sci. Med. Sci 66 (2011) 1090–1099. [PubMed: 21697501]

[28]. LeBrasseur NK, Bhasin S, Miciek R, Storer TW, Tests of muscle strength and physical function: reliability and discrimination of performance in younger and older men and older men with mobility limitations, J. Am. Geriatr. Soc 56 (2008) 2118–2123. [PubMed: 18811607]

[29]. Ware J, Kosinski M, SF-36 Physical and Mental Health Summary Scales: A Manual for Users of Version 1.2, Lincoln, RI, Quality Metric, 2005.

[30]. Cella D, Eton DT, Lai JS, Peterman AH, Merkel DE, Combining anchor and distribution-based methods to derive minimal clinically important differences on the Functional Assessment of Cancer Therapy (FACT) anemia and fatigue scales, J. Pain Symptom Manag 24 (2002) 547–561.

[31]. Derogatis L, Derogatis Affects Balance Scale (DABS): Preliminary Scoring, Procedures & Administration Manual. Clinical Psychometric Research, Baltimore, MD, 1996.

[32]. Fiatarone MA, Marks EC, Ryan ND, Meredith CN, Lipsitz LA, Evans WJ, High-intensity strength training in nonagenarians. Effects on skeletal muscle, JAMA 263 (1990) 3029–3034. [PubMed: 2342214]

[33]. Windhauser MM, Evans MA, McCullough ML, Swain JF, Lin PH, Hoben KP, Plaisted CS, Karanja NM, Vollmer WM, Dietary adherence in the dietary approaches to stop hypertension trial, DASH Collaborative Research Group, J. Am. Diet. Assoc 99 (1999) S76–S83. [PubMed: 10450298]

[34]. Delahanty LM, An expanded role for dietitians in maximising retention in nutrition and lifestyle intervention trials: implications for clinical practice, J. Hum. Nutr. Diet 23 (2010) 336–343. [PubMed: 20163510]

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Table 1

Eligibility criteria for the OTIMEN trial.

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 the score is 10, then the combination of scores must be 4-3-3)
3	Daily protein intake < 0.83 $g \cdot kg^{-1} \cdot day^{-1}$ (from the average of three 24-hour food recalls)
4	Able to give informed consent
Exclusion criteria	
1	History of prostate or breast cancer
2	Severe symptoms of benign prostatic hyperplasia as indicated by International Prostate Symptom Scale/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
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	Serum creatinine > 2.0 mg/dL
a.	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-melanoma skin cancers
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
12	Schizophrenia, bipolar disorder, or untreated diagnosed depression
13	TSH levels < 0.4 or > 7.5mLU/L
14	Systolic blood pressure (BP) > 160 or diastolic BP > 100 mmHg (average of 2 measurements taken at Visit 1)
15	Hemoglobin Ale > 8.0%
16	Subjects on insulin therapy will be excluded
17	Mini-Mental Status Exam (MMSE) score < 24
18	Body mass index (BMI) < 20 or > 40 kg/m^2
19	Not willing to eat red meat, fish, shellfish, poultry, or eggs
20	Allergy to peanuts, soy, sesame, shellfish, or gluten
21	Current alcohol use > 21 drinks/week based on self-report
22	Confinement to a wheelchair
23	Current use of any of the following medications: testosterone, DHEA, androstendione, rhGH, or levodopa within 1 year of screening
24	Current enrollment in a structured weight management program or participation in any weight intervention studies in the preceding 90 days
25	Serum ALT and AST > 3 × upper limit of normal
26	Hematocrit < 30% or > 48%
27	Progressive intensive resistance exercise training within 12 weeks of screening
28	Current use of anticoagulants
29	Noncompliant with run-in diet and/or study supplement (noncompliance is defined as intake of < 75% of the meals and supplements during the run-in period)

30	Unwillingness or inability to eat three frozen study meals daily for 6 months
31	Unwillingness to stop current nutritional supplements
32	Not appropriate for the study based on physician discretion

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Table 2

Efficacy outcomes.

Primary efficacy outcome
Whole body lean body mass measured using dual energy X-ray absorptiometry
Secondary efficacy outcomes
Appendicular skeletal muscle mass using DXA
Measures of muscle performance
Leg press strength
Chest press strength
Measures of Physical Function
6-minute walking distance and speed
Stair climbing speed and power
50-meter walking speed with and without carrying a 20% load
Self-reported physical function
Physical function domain of Medical Outcomes Study Short Form 36
Wellbeing, affectivity balance and fatigue
Wellbeing assessed using Psychological Wellbeing Index
Affectivity balance using DeRogatis Affectivity Balance Scale
Fatigue using the FACIT-1 scale

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Table 3

Schedule of events.

Procedure/Test	Screening	Baseline	Day 1	Week 6	Week 12	Week 18	Week 24
Consent / Eligibility	X						
24-hour food recalls	←	—	—	—	—	—	→
Randomization			X				
Bi-weekly food compliance checklists			←	—	—	—	→
Testosterone / Placebo injection			←	—	—	—	→
Protein / placebo supplements			←	—	—	—	→
CBC / chemistry panel / adverse event recording	X	X		X	X	X	X
24-hour urine for calcium, urea nitrogen		X			X		X
Physical exam / PSA / IPSS score / prostate exam	X	X		X	X	X	X
Body Composition by DXA		X			X		X
Muscle strength and leg power		X			X		X
Physical Function (Stair climbing, 6-min walk, load carry)		X			X		X
Self-reported physical function		X			X		X
Affectivity / fatigue/sense of wellbeing		X			X		X

CBC, complete blood count; PSA, prostate specific antigen; IPSS, International Prostate Symptom Scale; DXA, dual energy X-ray absorptiometry.

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Table 4

Safety outcomes.

Hemoglobin and hematocrit
PSA level
Lower urinary tract symptoms using International Prostate Symptom Score
Serum creatinine and blood urea nitrogen (BUN)
24-hour urinary calcium excretion
Blood chemistry panel