Title
The Testosterone Trials: Seven coordinated trials of testosterone treatment in elderly men

Journal
Clinical Trials, 11(3)

ISSN
1740-7745

Authors
Snyder, Peter J
Ellenberg, Susan S
Cunningham, Glenn R
et al.

Publication Date
2014-06-01

DOI
10.1177/1740774514524032

Peer reviewed

Published in final edited form as:

Clin Trials. 2014 June ; 11(3): 362–375. doi:10.1177/1740774514524032.

THE TESTOSTERONE TRIALS: THE DESIGN OF SEVEN COORDINATED TRIALS TO DETERMINE IF TESTOSTERONE TREATMENT BENEFITS ELDERLY MEN

Peter J Snyder, MD[1], **Susan S Ellenberg, PhD**[2], **Glenn R Cunningham, MD**[3], **Alvin M Matsumoto, MD**[4], **Shalender Bhasin, MD**[5], **Elizabeth Barrett-Connor, MD**[6], **Thomas M Gill, MD**[7], **John T Farrar, MD PhD**[8], **David Cella, PhD**[9], **Raymond C Rosen, PhD**[10], **Susan M Resnick, PhD**[11], **Ronald S Swerdloff, MD**[12], **Dr Jane A Cauley, PH**[13], **Denise Cifelli, MS**[8], **Laura Fluharty, MPH**[8], **Marco Pahor, MD**[14], **Kristine E Ensrud, MD MPH**[15], **Cora E Lewis, MD MPH**[16], **Mark E Molitch, MD**[17], **Jill P Crandall, MD**[18], **Christina Wang, MD**[12], **Matthew J Budoff, MD**[19], **Nanette K Wenger, MD**[20], **Emile R Mohler 3rd, MD**[21], **Diane E Bild, MD MPH**[22], **Nakela L Cook, MD MPH**[23], **Tony M Keaveny, PhD**[24], **David L Kopperdahl, PhD**[25], **David Lee, PhD**[25], **Ann V Schwartz, PhD MPH**[26], **Thomas W Storer, PhD**[27], **William B Ershler, MD**[28], **Cindy N Roy, PhD**[29], **Leslie J Raffel, MD**[30], **Sergei Romashkan, MD PhD**[31], and **Evan Hadley, MD**[31]

[1]Division of Endocrinology, Diabetes, and Metabolism, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

[2]Department of Biostatistics and Epidemiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

[3]Division of Diabetes, Endocrinology and Metabolism, Baylor College of Medicine and St. Luke's Episcopal Hospital, Houston, TX, USA

[4]Geriatric Research, Education, and Clinical Center, Division of Gerontology & Geriatric Medicine, Department of Veterans Affairs Puget Sound Health Care System, Department of Internal Medicine, University of Washington School of Medicine, Seattle, WA, USA

[5]Research Program in Men's Health: Aging and Metabolism, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

[6]Department of Family and Preventative Medicine, University of California San Diego School of Medicine, La Jolla, CA, USA

[7]Division of Geriatric Medicine, Yale School of Medicine, New Haven, CT, USA

[8]Center for Clinical Epidemiology & Biostatistics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

[9]Department of Medical Social Sciences, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Corresponding Author: Peter J. Snyder, MD.

Trial Registration: http://clinicaltrials.gov/ NCT00799617

Declaration of Conflict of Interest

[10]New England Research Institutes, Watertown, MA, USA

[11]Laboratory of Behavioral Neuroscience, National Institute on Aging, National Institutes of Health, Baltimore, MD, USA

[12]Division of Endocrinology , Harbor-University of California at Los Angeles Medical Center; Los Angeles Biomedical Research Institute ;Torrance, CA; David Geffen School of Medicine at University of California, Los Angeles, Los Angeles, CA, USA

[13]Department of Epidemiology, University of Pittsburgh, Graduate School of Public Health, Pittsburgh, PA, USA

[14]Department of Aging & Geriatric Research, University of Florida, Gainesville, FL, USA

[15]Department of Medicine, Division of Epidemiology & Community Health, University of Minnesota, Minneapolis VA Health Care System, Minneapolis, MN, USA

[16]Division of Preventive Medicine, University of Alabama at Birmingham, Birmingham, AL, USA

[17]Division of Endocrinology, Metabolism and Molecular Medicine, Northwestern University, Feinberg School of Medicine, Chicago, IL, USA

[18]Divisions of Endocrinology and Geriatrics, Albert Einstein College of Medicine, Bronx, NY, USA

[19]Division of Cardiology, Los Angeles Biomedical Research Institute, Harbor-University of California at Los Angeles Medical Center, Torrance, CA, USA

[20]Division of Cardiology, Emory Heart and Vascular Center, Emory University School of Medicine, Atlanta, GA, USA

[21]Division of Cardiovascular Disease and Section of Vascular Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

[22]Division of Cardiovascular Sciences, National Heart, Lung, and Blood Institute, Bethesda, MD, USA

[23]Clinical Applications and Prevention Branch, Division of Cardiovascular Sciences, Centers for Cardiovascular Outcomes Research, National Heart, Lung, and Blood Institute, Bethesda, MD, USA

[24]Departments of Mechanical Engineering and Bioengineering, University of California and ON Diagnostics, Berkeley, CA, USA

[25]O.N. Diagnostics, Berkeley, CA, USA

[26]Department of Epidemiology and Biostatistics, University of California San Francisco, San Francisco, CA, USA

[27]Section of Endocrinology, Diabetes, and Nutrition, Boston Medical Center, Boston, MA, USA

[28]Division of Hematology/Oncology, Institute for Advanced Studies in Aging, Falls Church, VA, USA

[29]Division of Geriatric Medicine and Gerontology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

[30]Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA

[31]Division of Geriatrics and Clinical Gerontology, National Institute on Aging, Bethesda, MD, USA

Abstract

Background—The prevalence of low testosterone levels in men increases with age, as does the prevalence of decreased mobility, sexual function, self-perceived vitality, cognitive abilities, bone mineral density, and glucose tolerance, and of increased anemia and coronary artery disease. Similar changes occur in men who have low serum testosterone concentrations due to known pituitary or testicular disease, and testosterone treatment improves the abnormalities. Prior studies of the effect of testosterone treatment in elderly men, however, have produced equivocal results.

Purpose—To describe a coordinated set of clinical trials designed to avoid the pitfalls of prior studies and determine definitively if testosterone treatment of elderly men with low testosterone is efficacious in improving symptoms and objective measures of age-associated conditions.

Methods—We present the scientific and clinical rationale for the decisions made in the design of this trial.

Results—We designed The Testosterone Trials as a coordinated set of seven trials to determine if testosterone treatment of elderly men with low serum testosterone concentrations and also symptoms and objective evidence of impaired mobility and/or diminished libido and/or reduced vitality would be efficacious in improving mobility (Physical Function Trial), sexual function (Sexual Function Trial), fatigue (Vitality Trial), cognitive function (Cognitive Function Trial), hemoglobin (Anemia Trial), bone density (Bone Trial), and coronary artery plaque volume (Cardiovascular Trial). The scientific advantages of this coordination were common eligibility criteria, treatment and monitoring and the ability to pool safety data. The logistical advantages were a single steering committee, data coordinating center and data safety monitoring board (DSMB), the same clinical trial sites, and the possibility of men participating in multiple trials. The major consideration in subject selection was setting the eligibility criterion for serum testosterone low enough to ensure that the men were unequivocally testosterone deficient, but not so low as to preclude sufficient enrollment or eventual generalizability of the results. The major considerations in choosing primary end points for each trial were identifying those of the highest clinical importance and identifying the minimum clinically important differences between treatment arms for sample size estimation.

Potential Limitations—Setting the serum testosterone concentration sufficiently low to ensure that most men would be unequivocally testosterone deficient, as well as many other entry criteria, resulted in screening approximately 30 men in person to randomize one subject.

Conclusions—The Testosterone Trials were designed to determine definitively if testosterone treatment of elderly men with low testosterone would have any clinical benefit. Designing The Testosterone Trials as a coordinated set of seven trials afforded many important scientific and logistical advantages but required an intensive recruitment and screening effort.

Keywords

clinical trial; testosterone; hypogonadism; aging; mobility; sexual function; vitality; cognitive function; anemia; CT angiography; volumetric BMD; bone strength

Introduction

The prevalence of low testosterone levels in men increases with age, as does the prevalence of many symptoms and conditions that are similar to those that occur when men of any age develop testosterone deficiency due to known pituitary or testicular disease. These parallels suggest the possibility that the lower testosterone levels in elderly men contribute to many other age-associated conditions. Prior trials of the effects of testosterone treatment of elderly men, however, yielded equivocal results. The Testosterone Trials are a coordinated set of clinical trials designed to determine if testosterone treatment would benefit elderly men who have low testosterone concentrations and conditions of which low testosterone might be a cause or a contributor.

Decline In Serum Testosterone With Aging In Men and Its Possible Consequences

Cross-sectional studies, such as the European Male Aging Study[1] and longitudinal studies, such as the Baltimore Longitudinal Study of Aging[2], showed gradual decreases in testosterone with increasing age. As men age, they also experience decreased muscle mass[3, 4] and mobility[5], decreased sexual function[6], decreased energy, anemia[7], decline in memory and other cognitive functions[8], decreased bone mineral density (BMD)[9] and increased fractures[10], increased fat mass[11] and impaired glucose tolerance[12].

Results of Prior Clinical Trials of Testosterone in Elderly Men

Several randomized, placebo-controlled trials studied the effects of testosterone treatment of elderly men with low-normal to slightly low serum testosterone concentrations. Testosterone treatment consistently increased lean mass and decreased fat mass[13-17] and tended to increase BMD of the spine[18, 19]. Effects on muscle strength and physical performance [13-15, 17, 20], sexual function[13, 17, 20], cognition[21, 22] and energy[13, 17, 21] were inconsistent.

The failure to document expected positive effects was variously attributed to selection of men who did not have sufficiently low testosterone concentrations and did not have abnormalities caused by low testosterone, insufficient increase of the serum testosterone concentration, use of insensitive outcome measures, and sample sizes too small for adequate power.

Institute of Medicine Report

In 2003, a panel of the Institute of Medicine (IOM) of the National Academy of Sciences concluded that the evidence at that time did not demonstrate any clear beneficial effect of testosterone treatment of elderly men and recommended a coordinated set of clinical trials to determine if such treatment would have any benefit[23]. The Testosterone Trials were designed to implement this recommendation.

Choice of Overall Study Design

The major feature of The Testosterone Trials design is a coordinated set of clinical trials to test the efficacy of testosterone treatment in elderly men who had a low serum testosterone

and one or more clinical abnormalities that low testosterone might cause. Each trial was designed to test the effect of testosterone on a clinical condition that testosterone is known to improve in men who have severe hypogonadism due to known disease of the pituitary or testes. The rationale for coordinating many aspects of these trials – including the recruitment, screening, treatment, and monitoring for safety, as well as the study governance and management – was to facilitate uniform selection and treatment and allow pooling of safety data.

Coordination also had two practical advantages. The first was enormous cost-efficiency, since the same recruiting, screening and study management was used for all trials. Costs were also reduced greatly because subjects could, if they qualified, participate in more than one trial, reducing the total number of subjects that had to be identified, screened and treated. The second was simplifying study management by having a single steering committee, data coordinating center and DSMB, and the same field sites for all trials (Appendix).

Subject Selection – Inclusion Criteria

The major factors considered in choosing inclusion and exclusion criteria for the common aspects of the trial (Table 1) were age, serum testosterone concentration, conditions that could interfere with the interpretation of the results, and conditions that testosterone treatment could exacerbate.

We chose 65 years as the lower age limit because it is a commonly used cutoff for studies of the elderly.

We used the serum total testosterone concentration for screening, even though it is the free testosterone that is biologically active, because assays for total testosterone are more accurate and better standardized than those for free, and usually total testosterone accurately reflects the free [24]. In addition, total testosterone assays are more widely available than free testosterone assays and more commonly used in clinical practice.

We selected a serum testosterone value for inclusion intended to be low enough that it would be considered unequivocally low but high enough that we would meet recruitment targets for statistical power. The lower limit of the normal range of total testosterone for young men is usually considered to be about 300 ng/dL early in the morning. Based on these considerations, we chose to include men whose serum testosterone was <250 ng/dL at 8-10AM. To account for spontaneous fluctuations, we required that subjects have this value on two separate visits. After six months of screening, however, only 11.6% of men had qualified by the first test and 67.1% of these by the second, so only 7.8% of subjects qualified by both. We therefore relaxed the initial value to <275 ng/dL, the second to <300 ng/dL, and the mean of the two to <275 ng/dL, still low enough to assure that the study subjects would be truly hypogonadal. As a result, by the 36th month of screening, an average of 20.6% of men had qualified by the first test and 68.1% of these by the second, or overall 14.0% by both.

Subjects were also required to have one or more symptoms that could be consequent to the low testosterone in order to qualify for one of the three main trials (see Main Trials, below).

Subject Selection – Exclusion Criteria

A final consideration in subject selection was to exclude men who were at relatively high risk of having conditions that testosterone treatment might exacerbate, such as prostate cancer, benign prostatic hyperplasia, erythrocytosis and sleep apnea.

Because metastatic prostate cancer regresses following medical or surgical castration[25] and is exacerbated following testosterone treatment[26], we excluded men who had a history of prostate cancer or prostatic intraepithelial neoplasia. We also excluded men who had a palpable prostate nodule by digital rectal examination. We further excluded men who had a relatively high risk of undiagnosed prostate cancer by the Prostate Cancer Risk Calculator[27], which takes into account the known risk factors of age, prostate specific antigen (PSA) concentration, race, family history, and a prior negative prostate biopsy.

Because PSA concentrations are lower in men with low testosterone than normal testosterone, we adjusted the PSA concentration used in this calculation to what it would have been had the subject's serum testosterone been normal, using unpublished data from the European Male Aging Study (courtesy of Dr. Frederick Wu) that showed a significant association between the serum PSA and the serum testosterone concentration in men ≥65 years whose serum testosterone was <460 ng/dL and PSA was <4 ng/mL. The regression coefficient was 0.00128 ($p = 0.007$), and the coefficient of determination (r^2) 0.016. The correction factor [(460 – serum testosterone concentration) × 0.00128] was added to the measured PSA to give the PSA value used in the risk calculator.

We excluded men whose risk of any prostate cancer was >35% and risk of high-grade cancer was >7%, values we considered conservative. For example, a 75 year-old Caucasian man who had no family history of prostate cancer could have had a serum PSA of no higher than 2.8 ng/mL to qualify by this criterion.

Benign prostatic hyperplasia is also dependent on testosterone and its conversion to dihydrotestosterone[28, 29]. We therefore also excluded men who had symptoms of severe lower urinary tract symptoms, as defined by a score of >19 on the International Prostate Symptom Score (IPSS) questionnaire.

Testosterone stimulates erythropoiesis, so a man who had a low testosterone and a hemoglobin concentration that was in the upper part of the normal range or higher likely could have had an underlying cause of erythrocytosis that testosterone treatment would have unmasked. We therefore excluded men who had hemoglobin concentrations >16.0 g/dL.

The evidence that testosterone exacerbates sleep apnea was inconclusive, but to be cautious, we excluded men with diagnosed but untreated sleep apnea.

After screening began but before the first subject was enrolled, a report of another testosterone trial described a large excess of cardiovascular events[30], so we excluded men

who had a myocardial infarction or stroke within the previous three months, systolic blood pressure >160 mm Hg, or diastolic blood pressure >100 mm Hg.

We also excluded men who had chronic illnesses of a severe degree that could limit performance on the test instruments but not be responsive to testosterone treatment (Table 1); men who had a serum testosterone concentration <100 ng/dL and a recognizable cause of primary or secondary hypogonadism; men who were taking a drug that affects the serum testosterone concentration; and men whose serum total testosterone concentration would not have accurately reflected their endogenous gonadal status because they had a high body mass index (BMI) (>37 kg/m^2), which lowers sex hormone binding globulin (SHBG) and thereby lowers the total testosterone but not the free testosterone.

Choice of Trials and Parameters

Prior evidence about which conditions of elderly men testosterone was most likely to ameliorate determined the choice of trials. The main trials were Physical Function, Sexual Function and Vitality. Cognitive function testing was performed on all subjects. The Anemia Trial included all subjects in the three main trials who were mildly to moderately anemic at baseline. The Cardiovascular and Bone Trials were open to subjects in the main trials who met additional specific entry criteria.

Treatment Allocation and Balancing

We allocated subjects to receive testosterone or placebo based on the covariate-adaptive approach of minimization. Minimization allows balancing with a larger number of variables than stratified randomization[31, 32]. Balancing variables included participation in each of the main trials, clinical site, mean screening testosterone concentration under or over 200, age under or over 75, current antidepressant use, and current PDE5 inhibitor use. We used the method of Taves[31], with the modification that treatment optimizing balance for each subject was assigned with 80% (rather than 100%) probability to maintain some randomness to the algorithm.

Testosterone Treatment

For a testosterone preparation, we chose a gel applied to the skin, because these preparations increase the serum testosterone concentrations of most hypogonadal men to within the normal range, and most men find them easier to use than injectable preparations. The National Institute on Aging issued a Request for Information (NOT-AG-05-005), asking for a donation of gel. Solvay Pharmaceuticals (later Abbott Laboratories, now AbbVie) offered AndroGel 1% and matching placebo.

The goal of testosterone treatment was to increase the serum testosterone concentration of the men assigned to the testosterone arm to within the normal range for young men and maintain it during the one-year of treatment. The initial dose was 5g of gel (containing 50 mg of testosterone). The serum testosterone concentration was measured at months 1, 2, 3, 6 and 9, and the dose of AndroGel was adjusted after each measurement, if necessary, to achieve that goal by an algorithm shown in Figure 1. The target range was initially 400-800 ng/dL, but was changed to 500-800 ng/dL after early trial data showed that the median

testosterone concentration was 400-500 ng/dL, lower than the middle of the target range. Compliance was assessed by weighing used and unused pump bottles. To maintain blinding when we changed the gel dose of a man in the testosterone arm because the serum testosterone was too high or low, we simultaneously changed the dose of a subject in the placebo arm who had recently had blood drawn.

Serum testosterone was measured in a central laboratory by liquid chromatography/tandem mass spectrometry. We monitored the performance of the assay in collaboration with the Testosterone Standardization Program of the Centers for Disease Control by inserting aliquots of high and low serum testosterone pools into the assay after every 60 subject samples.

Monitoring Subjects for Potential Adverse Effects of Testosterone

Subjects were monitored during the one-year of treatment for development of conditions that testosterone could exacerbate.

Subjects were monitored for the possible development of prostate cancer by performing a digital rectal examination and measuring the serum PSA concentration at 3 and 12 months. They were referred to a study urologist for consideration of a prostate biopsy if a nodule was detected or the PSA concentration increased by >1.0 ng/mL above the baseline value, confirmed by a second measurement. If the biopsy showed prostate cancer, gel treatment was discontinued.

Subjects were monitored for the possible development of severe lower urinary tract symptoms by administration of the IPSS at months 3 and 12. An increase to >19 resulted in a review of possible causes. If none was found, treatment with an alpha-adrenergic receptor blocking drug was considered. If symptoms persisted, the subject was referred to a urologist for evaluation.

Subjects were monitored for development of erythrocytosis by repeating the hemoglobin concentration at months 3, 6, 9 and 12. If the value increased to >17.5 g/dL, confirmed by a repeat test, a cause was sought. If none was found, the gel dose was reduced. If the value did not decrease to ≤17.5 g/dL, phlebotomy was recommended.

Subjects were monitored for the development of cardiovascular disease by asking at each visit about symptoms and diagnoses of specific cardiovascular diseases and procedures since the previous visit.

Specific Trials

To qualify for the TTrials overall, a man had to meet all of the general enrollment criteria described above and also the specific eligibility criteria for at least one of the three main trials (Table 1). A subject who also met the specific enrollment criteria for the Cardiovascular Trial and the Bone Trial could enroll in them. Each specific trial had its own hypotheses and tests of efficacy (Table 3). Most also had their own inclusion and exclusion criteria (Table 2).

Physical Function Trial

The primary hypothesis of the Physical Function Trial was that testosterone treatment for one year of elderly men with a low serum testosterone concentration and mobility disability would increase the proportion of men who improved their distance in the 6-minute walk test by ≥50m. The 6-minute walk test was selected as the primary outcome because 1) walking is essential for most activities of daily living and predicts meaningful clinical outcomes[33-35] and 2) data about the minimum clinically important difference (MCID) (50m) were available[36-38]. One investigator (TWS) trained the staff at each site in the administration of this test and visited each site once a year to monitor performance. This test was administered at months 0, 3, 6, 9 and 12. The sample size estimate was based on the MCID of 50m, the assumption that 15% of men in the placebo group and >30% of men in the testosterone group would increase by at least this amount, and the goal of 90% power. The estimated sample size was 175 per treatment arm.

The most important secondary hypothesis was that treatment would be associated with greater improvement in self-reported physical function, as assessed by the physical function component (PF10) of the Medical Outcomes Study Short Form-36 (MOS SF36) questionnaire[39].

The inclusion criterion for the Physical Function Trial was symptomatic and objective mobility disability, defined by a combination of self-reported difficulty walking one-quarter mile and/or walking up one flight of stairs and a gait speed of <1.2 meters/second on the 6-minute walk test, a value associated with reduced survival [4041]. Exclusion criteria are listed in Table 2.

Sexual Function Trial

The primary hypothesis of the Sexual Function Trial was that testosterone treatment for one year of elderly men with low sexual interest will increase sexual activity, as assessed by question 4 of the Harbor-UCLA 7-day Sexual Function Diary. This questionnaire had been used in two previous testosterone trials to demonstrate increased sexual activity [42, 43]. We administered it by interactive voice response (IVR) [44], but because it had not previously been administered by IVR, we first conducted a pilot study to compare the results obtained by IVR with those obtained by pencil and paper; the correlation (r) was 0.99, and Bland Altman analysis indicated excellent agreement (unpublished). The questionnaire was administered daily for seven days at months 0, 3, 6, 9 and 12. The sample size estimate was based on data that testosterone treatment of hypogonadal men increased the score by 0.75 units and a standard deviation of change from baseline to 12 months of 1.86 [43]. We calculated that 131 subjects/arm would provide 90% power to detect a change of this magnitude or greater.

Secondary end points included erectile function, assessed by the International Index of Erectile Function (IIEF), and libido, assessed by the Derogatis Inventory of Sexual Function - Male (DISF-M-II SR). Based on data showing that a change of 4 points in the IIEF is clinically meaningful [45] and that men who are not undergoing treatment do not experience a change, we estimated we would have 90% power to detect if there is a difference in the

proportion of men who improve their score by 4 or more points. We expect to have 80% power to detect a difference of 3 units in the DISF-M-II SR [46]

The inclusion criterion was decreased libido, both self-reported and by a score of ≤20 on the DISF-M-II SR questionnaire, as well as a partner willing to have sexual intercourse at least twice a month. Exclusion criteria are listed in Table 2.

Vitality Trial

The primary hypothesis of the Vitality Trial was that testosterone treatment for one year of elderly men with low vitality would increase the proportion whose score on the Functional Assessment of Chronic Illness Therapy (FACIT)-Fatigue scale increased by ≥4 [47]. This test was administered by IVR at months 0, 3, 6, 9 and 12. A pilot study showed a correlation (r) between IVR and paper versions of 0.92, and Bland Altman analysis indicated excellent agreement (unpublished). The sample size estimate was based on the assumptions that 20% of men in the placebo group and 35% of men in the testosterone group would exhibit an increase at least this great. We calculated that a sample size of 200 per treatment arm would provide 90% power to detect this difference.

Secondary outcomes included vitality, as measured by the SF-36 Vitality subscale, the two subscales of the Positive and Negative Affect Schedule (PANAS), and the Patient Health Questionnaire-(PHQ)-9 depression, which were also administered by IVR. A pilot study comparing the IVR and paper versions showed correlations (r) of 0.94 for the SF-36 Vitality, 0.96 for the PANAS, and 0.95 for the PHQ-9, and Bland Altman analyses indicated excellent agreement for all (unpublished).

The inclusion criterion for the Vitality Trial was low vitality, as defined by both self-reported decreased energy and a score of <40 on the FACIT-Fatigue scale. There were no exclusion criteria.

Cognitive Function Trial

The primary hypothesis was that testosterone treatment for one year of elderly men who have Age-Associated Memory Impairment (AAMI) would result in greater improvement, or less decline, in verbal memory, as assessed by the Delayed Paragraph Recall Subscale (Logical Memory II) of the Wechsler Memory Scale-Revised (WMS-R) [48]. Men with AAMI were defined as those who had subjective memory complaints, as determined by their score on the Memory Assessments Clinics-Questionnaire (MAC-Q), and objective memory impairment, as determined either by a score on the Delayed Paragraph Recall Subscale or by Benton Visual Retention Test (BVRT) >1 standard deviation (SD) below the performance for young men. We calculated that a sample size of approximately 235 men/arm would provide 90% power to detect a difference of 3 points in the Wechsler memory scale, the difference between the 50th percentile for 70-75 year old men (17) and that of 45-54 year-old men (20). Based on previous studies showing that about 60% of men over 65 years meet the criteria for AAMI, and an estimated total enrollment of 800, we anticipated having this power.

Secondary end points were visual memory, as assessed by the BVRT [49]; spatial ability, as assessed by the Card Rotation Test [50]; and executive function/working memory, as assessed by the Trail Making Test (TMT) [51].

The Wake Forest Cognitive Function Coordinating Center trained, certified and recertified semiannually the site personnel who administered the tests.

Anemia Trial

The primary aim, developed in collaboration with investigators of Partnership for Anemia: Clinical and Translational Trials in the Elderly (PACTTE), was to determine if testosterone treatment would improve unexplained mild to moderate anemia of the elderly, defined as a hemoglobin concentration 10.0-12.7 g/dL not due to nutrient deficiency, renal insufficiency, inflammation, or known hematologic disease. Specific exclusion criteria[52, 53] are listed in Table 2.

Based on data from the first 21 months of enrollment, we estimated that approximately 106 enrollees would have unexplained anemia of the elderly, which would provide 95% power to detect a difference of 1.0 g/dL in hemoglobin[54] between the two treatment groups at the end of 12 months of treatment.

Cardiovascular Trial

The primary hypothesis was that testosterone treatment would decrease progression of noncalcified coronary artery plaque volume, as assessed by computed tomography (CT) angiography. Data using similar CT angiography techniques to those employed in this trial indicated that scans at baseline and 12 months for 60 men/arm would be sufficient to give 80% power to detect a 13 mm^3 difference between treatment arms after one year of treatment, a value chosen to be more conservative than the 14 mm^3 treatment effect of statins (Dr. Matthew Budoff, unpublished data). Dr. Budoff trained the site CT technicians who performed the CT angiography.

There were no inclusion criteria. Exclusion criteria are listed in Table 2.

Bone Trial

The primary hypothesis was that testosterone treatment for one year would increase trabecular volumetric bone mineral density (vBMD) of the lumbar spine, measured by quantitative computed tomography (QCT). Assuming that testosterone increases vBMD by 9% in one year [55] and the standard error of the change is 3%, we estimated that we should have 90% power with 86 subjects per arm.

Secondary outcomes included trabecular vBMD of the hip, areal BMD (aBMD) of the spine and hip by dual energy absorptiometry (DXA), and bone strength of the spine and hip as determined by finite element analysis of the QCT data.

ON Diagnostics, the QCT Reading Center, trained the site CT technicians who performed the QCT scans and monitored scan quality. The University of California San Francisco DXA Quality Assurance Group trained the trial site DXA technicians and monitored scan quality.

There were no inclusion criteria. Exclusion criteria are listed in Table 2.

Lymphoblastoid Cell Lines

To allow for subsequent pharmacogenetic studies, lymphoblastoid cell lines were established by Epstein-Barr virus transformation[56] for all enrollees who consented. For controls, cell lines were also established on a subset of subjects at the first in person screening visit, most of whom had testosterone values >275 ng/dL.

Recruitment and Screening

Recruitment

After more than a year of recruiting using several methods, we found that a much greater number of enrollees came from mass mailings based on gender, age and zip codes, so subsequently we employed this method primarily.

Screening

Screening was conducted in three successive steps (Figure 2, Table 4):

Prescreening was conducted by telephone interview. Men who had a qualifying symptom and did not have a disqualifying disease by history were asked to come to the trial site for the first in-person screening visit.

At the first in-person screening visit, subjects had a more detailed medical history and had blood drawn for serum testosterone concentration. If the value was <275 ng/dL, a PSA and comprehensive metabolic panel were measured on saved serum. If the risk of prostate cancer, as determined by the Prostate Cancer Risk Calculator, was sufficiently low, the subject was asked to return for a second in-person screening visit.

At the second in-person screening visit, a second measurement of testosterone, digital rectal examination, and other tests were performed to determine if the subject qualified for any of the main trials. Subjects who qualified on the basis of these tests were asked to schedule a baseline visit.

Statistical Considerations

Treatment Blinding

Several methods were used to maintain blinding. Only the Data Coordinating Center and the Central Pharmacy knew treatment assignment. The testosterone and placebo preparations looked, smelled, and felt the same. When a subject in the testosterone arm was asked to change the dose of gel, a subject in the placebo group was asked to change his dose simultaneously. Clinical sites did not have access to laboratory tests, such as testosterone, PSA, and hemoglobin concentrations, that testosterone treatment might increase.

Sample Size Considerations

We collected primary endpoint data in the three main trials at baseline, 3, 6, 9 and 12 months. Therefore, sample size calculations assumed use of repeated outcome measures. To

be conservative, we performed sample size calculations based only on comparisons between baseline and 12-month values. We then inflated each sample size by 5% to compensate for subjects with no post-baseline measures.

Analytic Methods

Each of the trials is considered a separate trial, so we shall analyze the results of each trial separately. The primary and secondary endpoints of each trial will be evaluated for those subjects who qualified for that trial. Subjects will be analyzed in the group to which they were assigned, regardless of compliance with treatment. Each of the seven trials will be analyzed according to a prespecified analytic plan.

Primary analyses of outcomes from all time points will be performed with random effects models for longitudinal data. Logistic models will be used for binary variables and linear models for continuous variables. Outcomes with measures at baseline and 12 months only (Cardiovascular and Bone Trials) will be compared using multivariate logistic regression for binary variables and multivariate linear regression for continuous variables, where models adjust for balancing factors. Dichotomous outcomes, rather than continuous, will be used for analysis of MCIDs to determine not only if testosterone had a statistically significant effect compared with placebo, but also if it had an effect that was of clinical significance. We shall perform sensitivity analyses to assess the potential impact of missing data using shared parameter models, pattern mixture models, and inverse probability weighting. All analyses will be adjusted for baseline balancing factors.

Discussion

The goal of The Testosterone Trials was to determine if increasing the serum testosterone concentrations of elderly men with low levels to those of young men would have any clinical benefit. We considered this goal important for two main reasons. First, the fall in testosterone with increasing age and the parallels between the conditions of elderly men and those in men with low testosterone due to pituitary or testicular disease suggested that low testosterone might be a cause of many conditions of elderly men. However, prior trials of testosterone in elderly men had yielded largely equivocal results. We therefore considered it important to determine if testosterone had any beneficial effect. Second, at the time of the trials, testosterone use in elderly men was steadily increasing in the absence of evidence demonstrating its efficacy, so we considered it important to determine definitively if testosterone did not have a beneficial effect.

Many factors influenced the study design, especially the goals of selecting men who were unequivocally hypogonadal, setting power at 90% for sample size estimates, assessing functional end points of clinical importance, and coordinating the seven trials. In selecting subjects for the TTrials overall, the most important inclusion criterion was the serum testosterone concentration, because the goal of the study was to determine if raising the testosterone of elderly men with unequivocally low testosterone would benefit them. In addition, a reason often proposed for the equivocal results of prior studies was the inclusion of men whose concentrations were not unequivocally low. In determining the sample size for each of the seven trials, the most important choice was to set the power at 90% for detecting

a difference between treatment arms, so that negative as well as positive results would be considered definitive and have important implications for clinical practice. In choosing specific end points, we aimed for those that were clinically relevant rather than physiologically significant. We also chose differences between treatment arms that would be clinically meaningful.

Perhaps the most unusual aspect of the study design was conducting seven trials, each with its own primary, secondary, and exploratory end points, under the umbrella of a single trial. This structure had several advantages. The major scientific advantage was standardization of common entry criteria (e.g. serum testosterone concentration), testosterone treatment, and monitoring testosterone treatment. Because of this standardization, the results of the seven trials can be compared with each other more readily. The logistical advantage of this design was having one governing structure, one data coordinating center, one group of clinical trial sites, and one set of supporting teams. Corresponding to the logistical advantage was the financial advantage of having one set of each of the above. There was an even larger financial advantage of having a single group of subjects who could participate in more than one trial, since approximately half of the cost of the trial was for recruitment.

Allowing subjects to participate in more than one trial also had a disadvantage. Although a subject could, if he qualified and agreed, participate in all three main trials, as well as the Cardiovascular and Bone Trials, the number of tests that could be done in any one trial had to be limited to reduce subject burden.

The Testosterone Trials, in summary, are a highly coordinated group of seven randomized, placebo-controlled, multicenter trials designed to determine if testosterone treatment would benefit elderly men with low serum testosterone concentrations and conditions of which low testosterone might be a cause or a contributor. The advantages of the study design included common entry criteria, treatment and monitoring; the efficiency of having the same steering committee, data coordinating center, DSMB and trials sites; and the efficiency of subjects participating in more than one trial. We anticipate that The Testosterone Trials will provide definitive information about clinically meaningful benefits of testosterone treatment in these men.

Acknowledgments

The Testosterone Trials are supported by a grant from the National Institute on Aging, National Institutes of Health [U01 AG030644], supplemented by funds from the National Heart, Lung and Blood Institute. The Bone Trial is supported by a grant from the National Institute on Aging, National Institutes of Health [R01 AG037679]. The Anemia Trial is supported by a grant from the National Institute on Aging, National Institutes of Health [U01 AG034661] to the Partnership for Anemia Clinical and Translational Trials in the Elderly consortium. AbbVie (formerly Solvay and Abbott Laboratories) generously provided funding, AndroGel and placebo gel. AMM is supported by the Department of Veterans Affairs Puget Sound Health Care System. TMG is the recipient of a Midcareer Investigator Award in Patient-Oriented Research [K24 AG021507] from the National Institute on Aging. The Yale Field Center is partially supported by the Claude D. Pepper Older Americans Independence Center [P30 AG021342]. SMR is supported by the Intramural Research Program, National Institute on Aging, National Institutes of Health. CEL is supported by the National Institute for Diabetes, Digestive and Kidney Diseases, National Institutes of Health [DK079626] to the UAB Diabetes Research and Training Center. JAC is supported by the National Institute on Aging, National Institutes of Health [R01 AG37679]. LJR is supported by National Center for Research Resources [UL1 RR033176] and National Institute of Diabetes, Digestive and Kidney Diseases [P30 DK063491].

We thank many individuals who provided data prior to publication that aided greatly in the design of the TTrials, including Drs. Frederick Wu, David Lee, Eric Orwoll, Mary McDermott, Donald Brambilla, John McKinley, Donna Ankerst, and Kirk Esley. Many other individuals gave invaluable advice in the early stages of the design of the study, including Drs. Michael Barry, H. Ballantine Carter, Monique Cherrier, Suzanne Craft, Leonard Derogatis, Jerri Janowsky, Ira Katz, Scott Moffatt, and Ian Thompson. We thank Dr. Renee Moore and Yawei Zhang, Shannon Chuai and Liyi Cen for their support in developing the statistical aspects of the study design, and Dr. Kenneth Rockwell for designing the drug labeling and distribution processes. We thank Dr. Alisa Stephens for critical suggestions in drafting this paper. We thank Sandra Smith for excellent assistance with preparation of the manuscript.

References

1. Wu FC, Tajar A, Pye SR, et al. Hypothalamic-pituitary-testicular axis disruptions in older men are differentially linked to age and modifiable risk factors: the European Male Aging Study. J Clin Endocrinol Metab. 2008; 93:2737–45. [PubMed: 18270261]
2. Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. J Clin Endocrinol Metab. 2001; 86:724–31. [PubMed: 11158037]
3. Baumgartner RN, Koehler KM, Gallagher D, et al. Epidemiology of sarcopenia among the elderly in New Mexico. Am J Epidemiol. 1998; 147:755–63. [PubMed: 9554417]
4. Melton L 3rd, Khosla S, Crowson C, O'Connor M, O'Fallon W, Riggs B. Epidemiology of sarcopenia. Journal of the American Geriatrics Society. 2000; 48:625–30. [PubMed: 10855597]
5. Jette AM, Branch LG. The Framingham Disability Study: II. Physical disability among the aging. Am J Public Health. 1981; 71:1211–6. [PubMed: 7294262]
6. Bacon C, Mittlemen M, Kawachi I, Giovanucci E, Glasser D, Rimm E. Sexual function in men older than 50 years of age: Results from the health professionals follow-up study. Ann Intern Med. 2003; 139:161–8. [PubMed: 12899583]
7. Guralnik JM, Eisenstaedt RS, Ferrucci L, Klein HG, Woodman RC. Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. Blood. 2004; 104:2263–8. [PubMed: 15238427]
8. Price L, Said K, Haaland K. Age-associated memory impairment of Logical Memory and Visual Reproduction. J Clin Exp Neuropsychol. 2004; 26:531–8. [PubMed: 15512940]
9. Cawthon PM, Ewing SK, McCulloch CE, et al. Loss of hip BMD in older men: the osteoporotic fractures in men (MrOS) study. J Bone Miner Res. 2009; 24:1728–35. [PubMed: 19419308]
10. Drake MT, Murad MH, Mauck KF, et al. Clinical review. Risk factors for low bone mass-related fractures in men: a systematic review and meta-analysis. J Clin Endocrinol Metab. 2012; 97:1861–70. [PubMed: 22466344]
11. Forbes GB, Reina JC. Adult lean body mass declines with age: some longitudinal observations. Metabolism: clinical and experimental. 1970; 19:653–63. [PubMed: 5459997]
12. Cowie CC, Rust KF, Byrd-Holt DD, et al. Prevalence of diabetes and high risk for diabetes using A1C criteria in the U.S. population in 1988-2006. Diabetes care. 2010; 33:562–8. [PubMed: 20067953]
13. Snyder PJ, Peachey H, Hannoush P, et al. Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age. J Clin Endocrinol Metab. 1999; 84:2647–53. [PubMed: 10443654]
14. Page ST, Amory JK, Bowman FD, et al. Exogenous testosterone (T) alone or with finasteride increases physical performance, grip strength, and lean body mass in older men with low serum T. J Clin Endocrinol Metab. 2005; 90:1502–10. [PubMed: 15572415]
15. Kenny AM, Prestwood KM, Gruman CA, Marcello KM, Raisz LG. Effects of transdermal testosterone on bone and muscle in older men with low bioavailable testosterone levels. The journals of gerontology Series A, Biological sciences and medical sciences. 2001; 56:M266–72.
16. Blackman MR, Sorkin JD, Munzer T, et al. Growth hormone and sex steroid administration in healthy aged women and men: a randomized controlled trial. JAMA : the journal of the American Medical Association. 2002; 288:2282–92. [PubMed: 12425705]

17. Srinivas-Shankar U, Roberts SA, Connolly MJ, et al. Effects of testosterone on muscle strength, physical function, body composition, and quality of life in intermediate-frail and frail elderly men: a randomized, double-blind, placebo-controlled study. J Clin Endocrinol Metab. 2010; 95:639–50. [PubMed: 20061435]
18. Amory JK, Watts NB, Easley KA, et al. Exogenous testosterone or testosterone with finasteride increases bone mineral density in older men with low serum testosterone. J Clin Endocrinol Metab. 2004; 89:503–10. [PubMed: 14764753]
19. Snyder PJ, Peachey H, Hannoush P, et al. Effect of testosterone treatment on bone mineral density in men over 65 years of age. J Clin Endocrinol Metab. 1999; 84:1966–72. [PubMed: 10372695]
20. Liu PY, Wishart SM, Handelsman DJ. A double-blind, placebo-controlled, randomized clinical trial of recombinant human chorionic gonadotropin on muscle strength and physical function and activity in older men with partial age-related androgen deficiency. J Clin Endocrinol Metab. 2002; 87:3125–35. [PubMed: 12107212]
21. Kenny AM, Bellantonio S, Gruman CA, Acosta RD, Prestwood KM. Effects of transdermal testosterone on cognitive function and health perception in older men with low bioavailable testosterone levels. The journals of gerontology Series A, Biological sciences and medical sciences. 2002; 57:M321–5.
22. Vaughan C, Goldstein FC, Tenover JL. Exogenous testosterone alone or with finasteride does not improve measurements of cognition in healthy older men with low serum testosterone. J Androl. 2007; 28:875–82. [PubMed: 17609296]
23. Liverman, C.; Blazer, D. Testosterone and aging. Clinical research directions. The National Academies Press; Washington, DC: 2004.
24. Bhasin S, Cunningham GR, Hayes FJ, et al. Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2010; 95:2536–59. [PubMed: 20525905]
25. Crawford E, Eisenberger M, McLeod D, et al. A controlled trial of leuprolide with and without flutamide in prostatic carcinoma. The New England journal of medicine. 1989; 321:419–24. [PubMed: 2503724]
26. Fowler JJ, Whitmore WJ. The response of metastatic adenocarcinoma of the prostate to exogenous testosterone. J Urol. 1981; 126:372–5. [PubMed: 7277602]
27. Thompson IM, Ankerst DP, Chi C, et al. Operating characteristics of prostate-specific antigen in men with an initial PSA level of 3.0 ng/ml or lower. JAMA : the journal of the American Medical Association. 2005; 294:66–70. [PubMed: 15998892]
28. Roehrborn C, Boyle P, Nickel J, Hoefner K, Andriole G, on behalf of the Aria3001 A, and ARIA3003 Study Investigators. Efficacy and safety of a dual inhibitor of 5-Alpha-Reductase types 1 and 2 (Dutasteride) in men with benign prostatic hyperplasia. Urology. 2002; 60:434–41. [PubMed: 12350480]
29. Stoner E, The Finasteride Study Group. The clinical effects of a 5alpha-reductase inhibitor, finasteride, on benign prostatic hyperplasia. Journal of Urology. 1992; 147:1298–302. [PubMed: 1373779]
30. Basaria S, Coviello AD, Travison TG, et al. Adverse events associated with testosterone administration. The New England journal of medicine. 2010; 363:109–22. [PubMed: 20592293]
31. Taves D. Minimization: A new method of assigning patients to treatment and control groups. Clinical Pharmacology and Therapeutics. 1974; 15:443–53. [PubMed: 4597226]
32. Pocock S, Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. Biometrics. 1975; 31:103–15. [PubMed: 1100130]
33. Alexander N, Guire K, Thelen D, et al. Self-reported walking ability predicts functional mobility performance in frail older adults. Journal of the American Geriatrics Society. 2000; 48:1408–13. [PubMed: 11083316]
34. Guralnik JM, Ferrucci L, Pieper CF, et al. Lower extremity function and subsequent disability: consistency across studies, predictive models, and value of gait speed alone compared with the short physical performance battery. The journals of gerontology Series A, Biological sciences and medical sciences. 2000; 55:M221–31.

35. Jylha M, Guralnik J, Balfour J, Fried L. Walking difficulty, walking speed, and age as predictors of self-rated health: the women's health and aging study. J Gerontol A Biol Sci Med Sci. 2001; 56:M609–17. [PubMed: 11584033]
36. Kennedy D, Stratford P, Wessel J, Gollish J, Penney D. Assessing stability and change of four performing measures: a longitudinal study evaluating outcome following total hip and knee arthroplasty. BMC Musculoskeletal Disorders. 2005; 6
37. Perera S, Mody S, Woodman R, Studenski S. Meaningful change and responsiveness in common physical performance measures in older adults. Journal of the American Geriatrics Society. 2006; 54:743–9. [PubMed: 16696738]
38. Redelmeier D, Bayoumi A, Goldstein R, Guyatt G. Interpreting small differences in functional status: the Six Minute Walk test in chronic lung disease patients. Am J Respir Crit Care Med. 1997; 155:1278–82. [PubMed: 9105067]
39. Ware, J.; Kosinski, M. SF-36 Physical and Mental Health Summary Scales: A Manual for Users of Version 1. Second Edition. Quality Metric Inc.; Lincoln, RI: 2005.
40. Dumurgier J, Elbaz A, Ducimetière P, Tavernier B, Alpérovitch A, Tzourio C. Slow walking speed and cardiovascular death in well functioning older adults: prospective cohort study. BMJ. 2009; 339:b4460. [PubMed: 19903980]
41. Mutikainen S, Rantanen T, Alén M, et al. Walking ability and all-cause mortality in older women. Int J Sports Med. 2011; 32:216–22. [PubMed: 21165808]
42. Wang C, Swedloff RS, Iranmanesh A, et al. Transdermal testosterone gel improves sexual function, mood, muscle strength, and body composition parameters in hypogonadal men. Testosterone Gel Study Group. J Clin Endocrinol Metab. 2000; 85:2839–53. [PubMed: 10946892]
43. Steidle C, Schwartz S, Jacoby K, Sebree T, Smith T, Bachand R. North American AA2500 T Gel Study Group AA2500 testosterone gel normalizes androgen levels in aging males with improvements in body composition and sexual function. J Clin Endocrinol Metab. 2003; 88:2673–81. [PubMed: 12788872]
44. Kobak K, Greist J, Jefferson J, Katzelnick D, Mundt J. New Technologies to Improve Clinical Trials. Journal of Clinical Psychopharmacology. 2001; 21:255–6. [PubMed: 11386486]
45. Rosen RC, Allen KR, Ni X, Araujo AB. Minimal clinically important differences in the erectile function domain of the International Index of Erectile Function scale. European urology. 2011; 60:1010–6. [PubMed: 21855209]
46. Greenfield DM, Walters SJ, Coleman RE, et al. Quality of life, self-esteem, fatigue, and sexual function in young men after cancer: a controlled cross-sectional study. Cancer. 2010; 116:1592–601. [PubMed: 20186765]
47. 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 Manage. 2002; 24:547–61. [PubMed: 12551804]
48. Ferris S, Schneider L, Farmer M, Kay G, Crook T. A double-blind, placebo-controlled trial of memantine in age-associated memory impairment (memantine in AAMI). Int J Geriatr Psychiatry. 2007; 22:448–55. [PubMed: 17117395]
49. Benton, AL. Revised Visual Retention Test: Clinical and Experimental Applications. Manual. 4th ed.. The Psychological Corporation; New York: 1974.
50. R R. Validity of the trail making test as an indicator of organic brain disease. Perceptual Motor Skills. 1958; 8:6.
51. Ekstrom, RB., FJW; Harman, HH. Manual for Kit of Factor Referenced Cognitive Tests. Educational Testing Service; Princeton: 1976.
52. Price EA, Mehra R, Holmes TH, Schrier SL. Anemia in older persons: etiology and evaluation. Blood Cells Mol Dis. 2011; 46:159–65. [PubMed: 21208814]
53. Artz AS, Thirman MJ. Unexplained anemia predominates despite an intensive evaluation in a racially diverse cohort of older adults from a referral anemia clinic. J Gerontol A Biol Sci Med Sci. 2011; 66:925–32. [PubMed: 21659341]

54. Patrick DL, Gagnon DD, Zagari MJ, Mathijs R, Sweetenham J, Group EAS. Assessing the clinical significance of health-related quality of life (HrQOL) improvements in anaemic cancer patients receiving epoetin alfa. Eur J Cancer. 2003; 39:335–45. [PubMed: 12565986]

55. Katznelson L, Finkelstein JS, Schoenfeld DA, Rosenthal DI, Anderson EJ, Klibanski A. Increase in bone density and lean body mass during testosterone administration in men with acquired hypogonadism. J Clin Endocrinol Metab. 1996; 81:4358–65. [PubMed: 8954042]

56. Pressman S, Rotter J. Epstein-Barr virus transformation of cryopreserved lymphocytes: prolonged experience with technique. Am J Hum Genet. 1991; 49:467. [PubMed: 1651051]

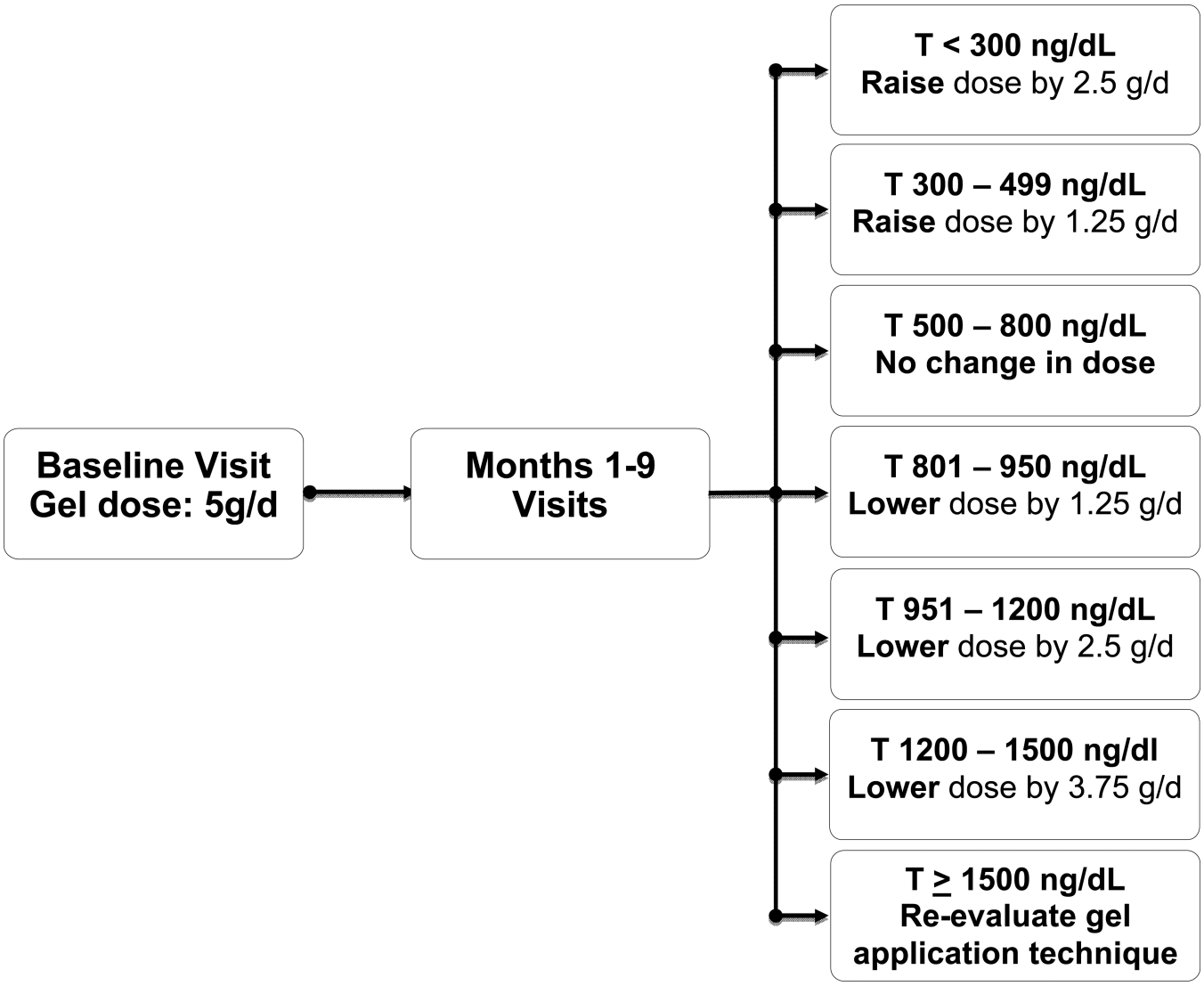

Figure 1.

Algorithm for raising or lowering the dose of testosterone gel based on the serum testosterone concentration of a man in the testosterone treatment arm at months 1, 2, 3, 6, and 9. Each time the dose of gel was changed in a man in the testosterone treatment arm, the dose was also changed in a man in the placebo arm.

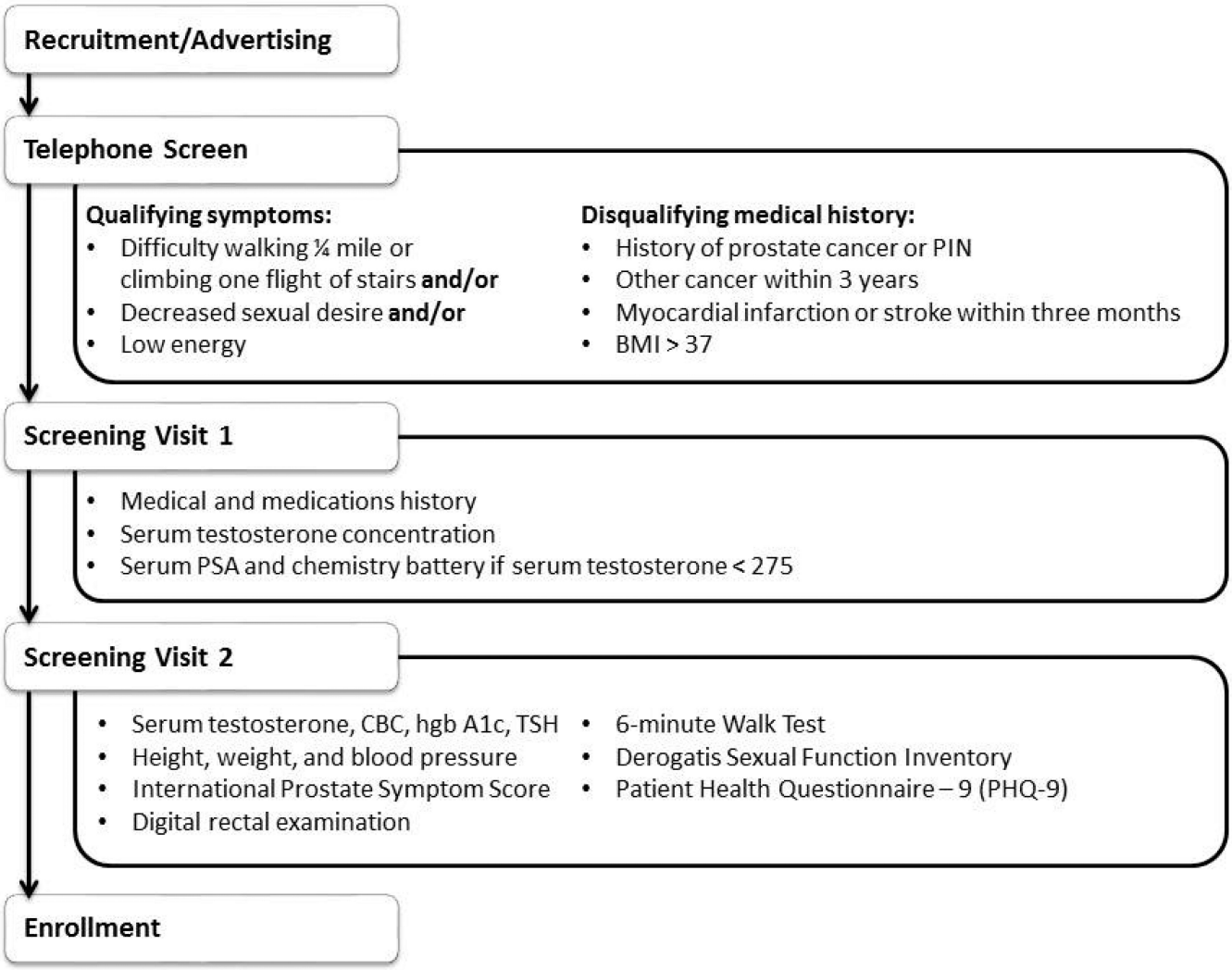

Figure 2.
The stepwise screening process of The Testosterone Trials from the telephone screen to enrollment.

TABLE 1

COMMON INCLUSION AND EXCLUSION CRITERIA FOR THE TESTOSTERONE TRIALS

Inclusion Criteria
• Men ≥65 years old
○ Total serum testosterone concentration at screening visit 1 (SV1) <275 ng/dL, at screening visit 2 (SV2) < 300 ng/dL and an average of both values < 275 ng/dL
Exclusion Criteria
• Diagnosed prostate cancer or prostatic intraepithelial neoplasia (PIN)
• Risk of prostate cancer by the Prostate Cancer Risk Calculator: >35% of overall prostate cancer or >7% risk of high grade prostate cancer
• Severe lower urinary tract symptoms (score of > 19) by the International Prostate Symptom Score questionnaire
• Hemoglobin <10 g/dL or >16.0 g/dL.
• Sleep apnea, diagnosed but untreated
• Alcohol or substance abuse within the past year (self-reported)
• New York Heart Association class III or IV congestive heart failure
• Myocardial infarction within the previous 3 months
• Angina not controlled by treatment
• Stroke within the previous 3 months
• Hypertension (systolic blood pressure >160 mm Hg or diastolic >100 mm Hg)
• Severe pulmonary disease precluding physical function tests
• ALT 3x upper limit of normal; hemoglobin A1c >8.5%
• TSH > 7.5 mIU/L
• Serum creatinine >2.2 mg/dL or renal disease requiring dialysis
• Diagnosis or treatment for cancer within the prior 3 years, with the exception of nonmelanotic skin cancers
• Body mass index (BMI) >37 kg/m^2
Exclusion Criteria
• Mini Mental State Exam (MMSE) Score <24
• Major psychiatric disorders that were untreated, unstable, or resulted in hospitalization or medication change in the prior three months or might have resulted in inability to complete the trial efficacy instruments. Subjects whose disorders had been stable while being treated for more than three months were eligible.
• Skin conditions at the gel application site that might affect testosterone absorption or tolerability of the gel
• Known skin intolerance to alcohol or allergy to any of the ingredients of testosterone gel
• Medications that affect serum testosterone concentration, (testosterone, androstenedione, DHEA, estrogens, GnRH analogs, spironolactone, and ketoconazole) for 2 months during the prior 12 months or within the prior three months.
• rhGH or megesterol acetate within the prior three months.
• Anti-depressant medication that had been introduced in the previous three months. (Subjects with diagnosed depression who had been stable for more than three months while taking anti-depressant medication were eligible.)
• Prednisone (dose >5 mg daily) use daily for more than two weeks, or equivalent doses of other glucocorticoids for more than two weeks during the previous three months.
• Opiate use within the prior three months, except intermittent use at doses that did not equal or exceed the equivalent of 20 mg methadone daily.

TABLE 2

INCLUSION AND EXCLUSION CRITERIA FOR SPECIFIC TRIALS IN THE TESTOSTERONE TRIALS

PHYSICAL FUNCTION TRIAL

Inclusion Criteria: symptomatic mobility disability, defined by

- Self-reported difficulty in walking one-quarter mile and/or self-reported difficulty in walking up one flight of stairs and
- Walking speed <1.2 meters/second on the 6-min walk test

Exclusion Criteria

- Not ambulatory
- Conditions affecting mobility of sufficient severity that testosterone was unlikely to improve, including progressive neurological conditions (multiple sclerosis) and severe, disabling arthritis of the legs or back

SEXUAL FUNCTION TRIAL

Inclusion Criteria

- Self-reported decreased libido
- Decreased libido, defined by a score of ≤20 on the DISF-M-II SR questionnaire
- A partner willing to have sexual intercourse ≥ twice/month

Exclusion Criteria

- Medical or nonmedical reasons that would preclude sexual activity (e.g., penile deformity, Peyronie's disease, pelvic surgery for bladder cancer)
- Severe peripheral vascular disease associated with an absence of pedal pulses
- Autonomic neuropathy

VITALITY TRIAL

Inclusion Criteria

- Self-reported decreased energy
- Low vitality, defined by a score <40 on the FACIT-Fatigue Scale

Exclusion Criteria: None

COGNITIVE FUNCTION TRIAL

- No specific inclusion or exclusion criteria.
- All subjects in all trials were administered all cognitive function tests

ANEMIA TRIAL

Inclusion Criteria

- Anemia (hemoglobin <12.7 g/dL)

Exclusion Criteria

- Iron deficiency (ferritin <50 ng/dL or transferrin saturation <20%)
- Folate deficiency (<3.4 ng/mL)
- B12 deficiency (<200 pg/mL)
- Inflammation (serum iron <60 μg/dL but failure to meet other criteria of iron deficiency)
- Mixed iron deficiency and inflammation (soluble transferrin receptor/log ferritin >2)
- Chronic renal insufficiency (eGFR <30 mL/min))
- Myelodysplastic syndrome (suspected by MCV >100 fL, platelet count <120 K/μL, or neutrophil count <1200 k/μL)
- Plasma cell dyscrasia (monoclonal gammopathy ≥1g/dL)
- Hemolytic anemia (normocytic or macrocytic anemia with elevated LDH and low haptoglobin)
- Thalassemia trait (MCV <80 fL and normal red blood cell count and iron)

CARDIOVASCULAR TRIAL

Inclusion Criteria: None

Exclusion Criteria

- eGFR <60 mL/min
- Weight >300 lb
- Allergy to iodinated contrast medium
- Atrial fibrillation

BONE TRIAL

Inclusion Criteria: None

Exclusion Criteria

- Bone mineral density of the lumbar spine, total hip or femoral neck by DXA T-score < −3.0
- Serum calcium >10.5 mg/dL
- Medications that could affect bone, including anticonvulsants, glucocorticoids (prednisone >20 mg/d > 2wk/yr or equivalent), bisphosphonates, teriparatide, or denosumab. Calcium was allowed.
- Surgery of the lumbar spine that prevented evaluation of at least one vertebra of L1-L4

TABLE 3

TESTS OF EFFICACY IN THE TESTOSTERONE TRIALS

Trials	Tests of Efficacy
Physical Function Trial	• 6-minute walk test (primary efficacy test)
	• Physical function scale (PF10) of the SF36
	• Patient global impression of change in walking a quarter mile
Sexual Function Trial	• Question 4 of the Harbor-UCLA 7-day Sexual Function Questionnaire (primary efficacy test)
	• Questions 1-3 and 5 and 6 of the Harbor-UCLA 7-day Sexual Function Questionnaire
	• Derogatis Sexual Function Inventory (Male) II (DSFI-M-II)
	• International Index of Erectile Function (IIEF)
	• Patient global assessment of change in sexual function
Vitality Trial	• FACIT-Fatigue scale (primary efficacy test)
	• Positive and Negative Affect Scale (PANAS)
	• Vitality scale of the SF-36
	• Patient Health Questionnaire (PHQ)-9 depression score
	• Patient global impression of change in fatigue/vitality
Cognitive Function Trial	• Wechsler Memory Scale-Revised, Logical Memory II (WMS-R LM II), delayed paragraph recall subtest (primary end point in men who have impaired memory at baseline)
	• Benton Visual Retention Test (BVRT)
	• Card Rotation Test
	• Trail Making Test (B-A score)
	• Patient global impression of change in memory
Anemia Trial	• Hemoglobin
Cardiovascular Trial	• CT Angiography
	• Fasting insulin and glucose, hemoglobin A1c
	• Total, HDL and LDL cholesterol
	• IL6, C-reactive protein
	• Endothelial microparticles
Bone Trial	• Quantitative CT-measured trabecular volumetric BMD of lumbar spine and hips
	• Quantitative CT-measured bone strength of the lumbar spine and hips
	• DXA-measured areal BMD of the lumbar spine and hips

Table 4

Testosterone Trial Test Schedule

PROCEDURES	Phone	Screen Visit 1	Screen Visit 2	Baseline	Treatment Month											Post-Rx	
					1	2	3	4	5	6	7	8	9	10	11	12	18	24
Screening & Monitoring																		
Symptom screen	X																	
Serum testosterone		X	X	X	X	X	X			X			X			X		
Efficacy trial screening			X															
PSA		X		X			X									X	X	
IPSS			X	X			X									X		
Urine sample stored			X	X	X	X	X			X			X			X		
Hct/Hgb			X	X			X			X			X			X		
History for clinical events	X			X			X			X			X			X	X	X
Medical history		X	X															
Digital rectal examination			X				X									X		
Cardiovascular history				X														
CV events and symptoms					X	X	X			X			X			X	X	X
Primary Efficacy Endpoints																		
Physical function				X			X			X			X			X		
Sexual function				X			X			X			X			X		
Vitality				X			X			X			X			X		
Cognition				X						X						X		
Secondary Efficacy Endpoints																		
Physical function				X			X			X			X			X		
Sexual function				X			X			X			X			X		
Vitality				X			X			X			X			X		
Cognition				X						X						X		
Other Efficacy Area																		
Anemia				X			X			X			X			X		
Measures Across Trials																		

PROCEDURES	Phone	Screen Visit 1	Screen Visit 2	Baseline	Treatment Month												Post-Rx	
					1	2	3	4	5	6	7	8	9	10	11	12	18	24
PGIC				X			X			X			X			X		
Falls				X			X			X			X			X		
PANAS				X			X			X			X			X		
PHQ-9				X			X			X			X			X		

Abbreviation: IPSS, International Prostate Symptom Score; PANAS, Positive and Negative Affect Scales; PGIC, Patient Global Impression of Change; PHQ-9, Patient Health Questionnaire-9