Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Basaria S, Coviello AD, Travison TG, et al. Adverse events associated with testosterone administration. N Engl J Med 2010;363:109-22. DOI: 10.1056/NEJMoa1000485.

Supplementary Appendix

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A. Detailed Methods

Study Design

The TOM Trial was a parallel group, randomized, placebo-controlled, double-blind trial in community–dwelling men, approved by institutional review boards of Boston University Medical Center (BUMC), New England Research Institutes (NERI), and the VA Boston Administration Healthcare System (VAHCS). All participants provided written, informed consent. Recruitment took place at VAHCS, NERI, and BUMC. Outcome assessments were performed at BUMC. The study consisted of a 24-week intervention followed by a 12-week observation period. Enrollment took place between September 2005 and December 2009.

A Data and Safety Monitoring Board (DSMB), established by the National Institute on Aging reviewed the study protocol in March 2005, and study progress and safety data every six months in 2005, 2006, 2007, and 2008 and four times in 2009 leading up to its meeting on December 31, 2009 in which it decided to stop further enrollment and administration of study medication based on the evaluation of adverse events in relation to potential benefits of the study medication.

Eligibility Criteria

Participants were men, ≥65 years old, with total testosterone 100-350ng/dL or free testosterone <50pg/mL in a sample drawn between 7 and 11AM. The subjects were defined as *mobility limited* if they reported difficulty walking two blocks on a level surface or climbing ten steps and had Short Physical Performance Battery (SPPB) scores between 4 and 9 representing mild to moderate mobility limitation.[1] Exclusion criteria included prostate or other active cancers, American Urological Association

(AUA) symptom score>21, systolic (SBP) or diastolic blood pressure (DBP) >160 or >100 mm Hg, respectively, unstable angina, myocardial infarction within 3 months of enrollment, untreated severe obstructive sleep apnea, current glucocorticoid use, use of any anabolic therapies in the preceding year, PSA>4ng/ml, alanine or aspartate aminotransferase >3-times the upper limit of normal, creatinine>3.5 mg/dL, hemoglobin A1c>8.5%, hematocrit>48%, body mass index (BMI)>40 kg/m^2, and class III or IV congestive heart failure. We excluded men who had mobility-limiting neuromuscular, joint or bone disease. Eligibility was verified by structured interviews/questionnaires, review of medical records, physical examination, and laboratory tests.

Randomization and Blinding

Eligible subjects were stratified by age (65-75 and >75) and randomized to placebo or testosterone gel based on concealed, computer-generated randomization tables, using a block size of 6. Study personnel and participants were blinded to randomization assignment.

Intervention

Participants applied transdermal gel containing either placebo or 10-g gel containing 100-mg testosterone (Testim 1%, Auxilium Pharmaceuticals, Norristown, PA) daily for 6 months. This dose was selected because in pivotal trials in hypogonadal men, this dose raised serum testosterone concentrations into the middle of the range for healthy young men[2]. Two weeks after randomization, testosterone levels were measured two- and four-hours after gel application. If average testosterone concentration was <500ng/dl or >1000ng/dL, the dose was increased to 15-g or reduced to 5-g. All subjects received three gel tubes daily in various combinations of

placebo and testosterone to maintain blinding. The subjects were provided written instructions to apply the gel on the upper arm and shoulder area, wash hands after gel application, and to keep the area of gel application covered with clothing to prevent transfer to another person who might come in intimate contact.

Outcomes

The primary efficacy outcome was change from baseline in maximal voluntary muscle strength in leg press exercise using one-repetition maximum (1RM).[3,4] Secondary efficacy outcomes included changes in chest press strength, 50-m walking speed, and stair climbing speed and power, and lift-and-lower test. These measures were performed at baseline and week 24.[3] End-of-study assessments were performed at the last visit if possible when study medication was discontinued before six months.

Testosterone was measured at Quest Diagnostics, San Juan Capistrano, CA using Bayer-Advia-Centaur immunoassay that has excellent correlation with liquid chromatography tandem mass spectrometry (r^2=0.945)[5] and sensitivity of 10ng/dL. SHBG was measured by an immunofluorometric assay (Delphia-Wallac, Inc) with sensitivity 2.5nmol/L[6]. Free testosterone was calculated.[7]

Assessment of Muscle Performance and Physical Function

Maximal Voluntary Muscle Strength. Seated leg press and chest press strength was determined using pneumatic resistance machines (Keiser Sport, Fresno, CA) and the one-repetition maximum (1-RM) method as previously reported[3,4]. Briefly, subjects completed a 5 min warm up including low resistance repetitions of each specific exercise. Resistance was increased progressively with 1-2 min rest between sets as attempts were made to achieve 1-RM, the maximum amount of resistance that could be

moved once only. All tests were repeated on a non-consecutive day within 7 days of the initial test to ensure reliability. We have previously demonstrated the reliability of the 1RM method in older men with mobility limitations (intra-class correlation coefficient >0.983)[3].

Tests of Physical Function. A 50-m walk, 12-step stair climb, and lift and lower task were used to assess functional performance; the details of these tests have been previously described[3]. Briefly, subjects completed two 50-m walk tests with and without carrying a load equal to 20% of their body weight. Two trials were given for each test with the subjects completing the course as quickly as possible without running. Walking speed was recorded using a switch mat and infrared timing system (Lafayette Instrument Company, Lafayette, IN) with the fastest time used as the criterion measure. Assistive devices (e.g., canes or walkers) were permitted. Both unloaded and loaded 50-m walk tests demonstrate excellent reliability (ICCs 0.988 and 0.991, respectively).

The stair climb tests required subjects to complete two ascents of a 12-step staircase as quickly as possible while touching each step. The second test required subjects to climb the same staircase but with the addition of carrying weighted tote bags holding 20% of their body weight. Subjects unable to complete the test safely were excluded from the analysis. Time was recorded by an electronic timing system (Lafayette Instrument Company, Lafayette, IN) interfaced with switch mats placed at the base of the stairs and at the twelfth step. Stair climbing power was calculated as the product of body weight plus weight carried, the total stair rise (2.04 m), and the acceleration of gravity all divided by ascent time. The intra-class correlation coefficients for unloaded and loaded stair climb test are 0.992 and 0.978, respectively[3], consistent with high degree of reliability.

Subjects also completed two trials of lifting and lowering a basket holding weight plates equivalent to 15% body weight. The test started with the weighted basket positioned at standard desk height (78.5 cm). At the start signal, subjects lifted the basket to a shelf at shoulder height thence to a shelf at head height. The sequence was then reversed and repeated as many times as possible in 1 minute. The number of shelves completed as well as the total weight moved (shelves multiplied by weight) was recorded. Two min rest was given between trials. The intra-class correlation coefficient for lift and lower test in older men is 0.947[3].

Safety Monitoring

Hemoglobin, hematocrit, PSA, and blood chemistries were monitored at baseline and 6, 12, 18, 24, and 36 weeks post-randomization. Subjects were asked about AEs and adherence at baseline and weeks 2, 6, 12, 18, 24, and 36. Prostate examination and AUA symptom scores were recorded at baseline and weeks 12, 24, and 36.

AEs were categorized by KAI Research, Rockville, MD using the Medical Dictionary for Regulatory Affairs (MedDRA) System Organ Class (SOC). The DSMB reviewed serious AE (SAE) reports as they occurred and cumulative AE reports every 6-months. In response to an imbalance in cardiac events between groups, the DSMB reviewed unblinded data in December 2009 and requested analyses of two additional categories in addition to the MedDRA-classified cardiac events. The first category included MedDRA-classified cardiac events plus events which the DSMB considered to be cardiovascular in nature but which were included in other MedDRA SOC categories: “Surgical and Medical Procedures” (stenting procedures and coronary artery bypass graft surgery), "General Disorders" (peripheral edema), “Investigations” (elevations of

blood pressure, arrhythmias, or ECG changes), and "Nervous System Disorders" (stroke and syncope). For clarity, these AEs are listed as "cardiovascular-related events". The second analysis included "atherosclerosis-related events" which were more directly related to atherosclerotic vascular disease such as myocardial infarction, sudden death, angioplasty, coronary bypass graft surgery, and stroke. Myocardial infarction, sudden death, stroke, and interventional procedures such as angioplasty and coronary artery bypass grafting were verified from medical records. Edema, blood pressure elevations, arrhythmias, and ECG changes were verified by examination by a study physician. Transient loss of consciousness and fainting were recorded as syncope.

Statistical Analyses

Although the study's planned principal aim was to determine testosterone's effects on muscle strength and physical function, the primary analysis in this report is an assessment of the incidence and prevalence of AEs among 209 subjects randomized as of December 15, 2009. The planned sample size of 252 randomized men was based on assumption of type I error probability of 0.05, statistical power of 90%, a testosterone-associated increase of 245 N (25 kg) in bilateral leg press strength and a standard deviation of treatment effect of 540 N (55 kg) based on previous research[6], and 20% loss to follow-up. At the time of the AE analyses as of December 15, 2009, 209 men had been randomized.

The proportion of subjects with $\geq$1 AE was compared between groups using chi-squared and Fisher's exact tests. Relative propensities for reporting $\geq$1 AEs were presented as odds ratios with 95% confidence intervals obtained by logistic regression,

unadjusted and adjusted for age group, BMI, diagnoses of diabetes, hyperlipidemia, and hypertension, smoking, and HDL cholesterol (HDLc).

The time to report of the first AE was computed for three categories: cardiovascular-related, dermatologic, and referral for medical evaluation. Subjects with no AEs were considered censored on the earliest of: the day of last recorded study visit, dropout date, or December 15, 2009. The proportion of subjects experiencing an AE was computed as a function of time using the Kaplan-Meier method stratified by treatment group, and compared using log rank tests. Cox proportional hazards regression models were used to estimate the relative likelihood of incident AEs with time.

Due to trial cessation, analyses of efficacy data were restricted to subjects with an end-of-study outcome measurement. Change scores were computed by subtracting baseline from end-of-study value. The group means were compared using two-sample Student's t-tests without assumption of equal variances, employing the Satterthwaite approximation of test degrees of freedom.

Sensitivity Analyses

A post-hoc analysis of cardiovascular AEs was performed to assess the sensitivity of conclusions to the removal of subjects with poorly controlled hypertension, screening testosterone<100 ng/dL, and those considered not to conform to planned eligibility criteria. The exclusion criterion of SBP>160 or DBP>100 was operationally defined during the study as the consistent finding of SBP>160 or DBP>100 mm Hg at all screening measurements. To obtain a more precise estimate of mean blood pressure during the screening phase, all blood pressure measurements taken during the

screening phase were averaged. Nineteen subjects with average SBP>160 or DBP>100 mm Hg were excluded. Additionally, two subjects with total testosterone<100ng/dl, one with hematocrit >48%, one with HbA1C >8.5, one with MMSE <24, two with depressive illness, and one with sleep apnea were excluded. The sensitivity analyses were conducted by applying the above models to the remaining 182 subjects, 90 on testosterone and 92 on placebo.

A second sensitivity analysis considered cardiovascular related adverse events excluding subjects with baseline evidence of pre-existing cardiovascular disease . A total of 104 (50%) participants had self-reported cardiovascular, peripheral vascular, or cerebrovascular disease, aortic aneurysm, congestive heart failure (CHF), or arrhythmias at baseline.

A total of 41 participants did not report difficulty in walking or climbing stairs, and 1 had a score on the Short Physical Performance Battery that was outside the eligibility range. Therefore, a third sensitivity analysis considered baseline self-reported mobility status and SPPB scores as potential confounders.

B. RESULTS OF THE SENSITIVITY ANALYSES

In the first sensitivity subsample (n=182), the unadjusted odds ratio (95% CI) for atherosclerotic events was 7.7 (0.9, 63.6) and for cardiovascular related events was 6.4 (1.8, 22.9). Adjusting for self report of baseline mobility disability and baseline SPPB score, the odds ratio for cardiovascular related events was 6.2 (1.7, 22.3).

In the second set of analyses, the 104 (50%) participants who had self-reported cardiovascular, peripheral vascular, or cerebrovascular disease, congestive heart failure (CHF), and arrhythmias at baseline were excluded. Of the remaining 105 subjects, nine men receiving testosterone had cardiovascular-related events as compared to two men receiving placebo gel (OR 5.8 (95%CI 1.2, 28), p=0.03); that is, results were essentially unchanged. Three men in the testosterone group had MedDRA cardiac events and two had atherosclerotic events – all in the testosterone group vs zero each in the placebo group.

In the final set of analyses, controlling for baseline mobility status or SPPB scores had no effect on results.

C. SUPPLEMENTARY APPENDIX TABLES

Supplementary Appendix Table 1. Change in laboratory values from baseline to last available treatment phase follow-up.

	Testosterone Mean (95% CI)	Placebo Mean (95% CI)	P
[a]**Total Testosterone (ng/dL)**	328 (239, 418)	54 (26, 83)	<0.0001
[a]**Free Testosterone (pg/mL)**	61 (40, 82)	0.2 (-4.1, 4.5)	<0.0001
Hemoglobin (g/dL)	0.8 (0. 5, 1.0)	-0.05 (-0.2, 0.1)	<0.0001
Hematocrit (%)	3.0 (2.3, 3.8)	0.01 (-0.6, 0.6)	<0.0001
Total Cholesterol (mg/dL)	-10.8 (-17.7, 3.9)	-3.4 (-8.5, 1.6)	0.09
HDL Cholesterol (mg/dL)	-1.1 (-2.6, 0.5)	2.9 (0.7, 5.1)	0.004
Triglycerides (mg/dL)	-15.5 (-30.7, -0.2)	-14.4 (-26.2, -2.6)	0.91
LDL Cholesterol (mg/dL)	-4.2 (-9.6, 1.2)	2.6 (-1.1, 6.3)	0.04
PSA (ng/mL)	0.3 (0.1, 0.4)	0.1 (-0.03, 0.2)	0.06
Platelets (1000s/µl)	2.9 (-10.0, 15.8)	-5.9 (-12.1, 0.4)	0.23
AST (U/L)	0.8 (-0.7, 2.3)	-0.4 (-1.6, 0.7)	0.19
ALT (U/L)	-0.1 (-2.4, 2.3)	-0.9 (-2.2, 0.5)	0.57

N = 179 (89 and 90 subjects, respectively, in the Testosterone and Placebo arms, with follow-up total and free testosterone measurements available on 81 (91%) and 86 (96%)). Testosterone levels were not available on subjects who dropped out or were discontinued prior to week 12 visit. Computations are performed on nonmissing records; fewer than 8% data are missing in any other row.

To convert serum testosterone level in ng/dL to nmol/L units, multiply serum testosterone in ng/dL with 0.0347. To convert free testosterone from pg/mL to pmol/L, multiply free testosterone in pg/mL with 3.47.Baseline testosterone was measured in a single sample drawn between 7 and 11 AM. Post-randomization testosterone level was measured in two blood samples drawn 10 minutes apart and pooled.

Supplementary Appendix Table 2. Number and proportion of subjects reporting adverse events, by category

	Testosterone	Placebo	P
Number of Subjects	n = 106	n = 103	
Blood and lymphatic disorders	0 (0%)	0 (0%)	--
Cardiac disorders[a,c]	10 (9%)	1 (1%)	0.006 [b]
Cardiovascular-related events	23 (22%)	5 (5%)	< 0.001
Atherosclerotic events	7 (7%)	1 (1%)	0.07[b]
Ear and labyrinth disorders	3 (3%)	0 (0%)	0.25 [b]
Endocrine disorders	1 (1%)	1 (1%)	1.0 [b]
Eye disorders	4 (4%)	2 (2%)	0.68 [b]
Gastrointestinal disorders	10 (9%)	9 (9%)	0.86
General disorders and administration site conditions	11 (10%)	8 (8%)	0.51
Hepatobiliary disorders	0 (0%)	1 (1%)	0.49 [b]
Immune system disorders	0 (0%)	1 (1%)	0.49 [b]
Infections and infestations	18 (17%)	16 (16%)	0.78
Injury, poisoning and procedural complications	15 (14%)	9 (9%)	0.22
Investigations	14 (13%)	7 (7%)	0.12
Metabolism and nutrition disorders	5 (5%)	1 (1%)	0.21 [b]
Musculoskeletal and connective tissue disorders	32 (30%)	20 (19%)	0.07
Neoplasms benign, malignant and unspecified (includes cysts and polyps)	3 (3%)	5 (5%)	0.49 [b]
Nervous system disorders	10 (9%)	7 (7%)	0.49
Psychiatric disorders	5 (5%)	3 (3%)	0.72 [b]
Renal and urinary disorders	7 (7%)	8 (8%)	0.74
Reproductive system and breast disorders	6 (6%)	3 (3%)	0.50 [b]
Respiratory, thoracic and mediastinal disorders[c]	8 (8%)	1 (1%)	0.04 [b]
Skin and subcutaneous tissue disorders[c]	19 (18%)	8 (8%)	0.03
Surgical and medical procedures	6 (6%)	3 (3%)	0.50 [b]
Vascular disorders	3 (3%)	1 (1%)	0.62 [b]
Number Referred for Medical Evaluation due to AE	19 (18%)	9 (9%)	0.05
Number of Serious AEs	16 (15%)	8 (8%)	0.10
Number of Life Threatening AEs	5 (5%)	1 (1%)	0.21 [b]
Total number reporting AEs	85 (80%)	65 (63%)	0.006

[a]Adverse events (AEs) were classified by KAI Research, Inc, Rockville, MD according to the MedDRA classification system, except for "cardiovascular-related events" and "atherosclerotic events" which were classified as described in the Methods section.

[b] Comparison by Fisher's Exact Test; All other comparisons by chi-squared test

[c] "Respiratory, thoracic and mediastinal disorders" included cough, asthma exacerbation, shortness of breath, sinus problems, exacerbation of COPD, and sleep apnea. 'Skin and subcutaneous tissue disorders' included skin reactions at gel application site, itching, erythema, psoriasis, foot ulcers, and increased hair growth. 'Cardiac disorders' included myocardial infarction, acute coronary syndrome, arrhythmias including atrial fibrillation and supraventricular tachycardia, congestive heart failure, and sudden death.

Supplementary Appendix Table 3: Serious Adverse Events and Life Threatening Adverse Events in the TOM Trial

	SERIOUS ADVERSE EVENTS	MedDRA SOC Classification (28 events in 24 men)
Testosterone Group		
1	Acute coronary syndrome	Cardiac Disorders
2	Diabetic foot ulcer	Metabolism & Nutrition Disorders
3	Prostate cancer	Investigations
4	Cholecystitis	Infections & Infestations
5	Myocardial infarction treated with angioplasty, pacemaker placement	Cardiac Disorders
6	Myocardial infarction	Cardiac Disorders
7	Panic attack	Psychiatric Disorders
8	Appendicitis	Infections & Infestations
9	Angioplasty and coronary artery bypass grafting	Surgical & Medical Procedures
10	Congestive heart failure	Cardiac Disorders
11	Fracture of the tibia	Injury, Poisoning & Procedural Complications
12	Stroke	Nervous System Disorders
13	Death, suspected myocardial infarction	Cardiac Disorders
14	Bupropion toxicity	Nervous System Disorders
15	Peripheral angioplasty and stenting	Surgical & Medical Procedures
16	Clozapine toxicity	Injury, Poisoning & Procedural Complications
17	Congestive heart failure exacerbation	Cardiac Disorders
18	Community acquired pneumonia	Infections & Infestations
Placebo Group		
1	Syncope resulting in hospitalization	Nervous System Disorders
2	Painful neuropathy	Nervous System Disorders
3	Hepatocellular carcinoma	Hepatobiliary Disorders
4	Intestinal obstruction	Gastrointestinal Disorders
5	Transurethral resection of prostate	Surgical & Medical Procedures
6	Abdominal pain	Gastrointestinal Disorders
7	Splenic rupture	Injury, Poisoning & Procedural Complications
8	Inflammatory colonic mass related to Crohn's Disease	Neoplasms: Benign, Malignant & Unspecified
9	Rectal bleeding from Crohn's Disease	Gastrointestinal Disorders
10	Community Acquired Pneumonia	Infections & Infestations

Legend: Life threatening events are shaded in gray (5 in the testosterone group and 1 in the placebo group). A Serious Adverse Event was defined according to the Food and Drug Administration as an adverse event that: 1) resulted in death, 2) was life threatening, 3) resulted in inpatient hospitalization or prolongation of hospitalization, 4) resulted in a persistent or significant disability/incapacity, or 5) based upon appropriate medical judgment, jeopardized the subject's health and required medical or surgical intervention to prevent one of the other outcomes listed in this definition. A Life Threatening Event was any adverse event that placed the subject at immediate risk of death from the event as it occurred.

Supplementary Appendix Table 4. Testosterone thresholds used as the inclusion criterion, testosterone doses, and on-treatment serum testosterone levels in testosterone trials in middle-aged and older men.

Study	Age for inclusion or age range of included men (Mean age)	Testosterone Threshold for Eligibility	Testosterone Formulation	Testosterone Dose	On-Treatment Mean Testosterone Level
The TOM Trial	≥ 65 (74)	Total T 100-350 ng/dL or free T <50 pg/mL	Transdermal 1% testosterone gel	Starting dose 10 g daily; dose adjusted based on week 2 T level	574 ng/dL
Agledahl 2008[8]	60-80 (~69)	Total T <315 ng/dL	Testosterone undecanoate injections	1000 mg every 12 weeks	Not provided
Allan, 2008[9]	≥55 (~63)	Total T <430 ng/dL	Transdermal testosterone patch	5 mg daily	~500 ng/dL
Basurto 2008[10]	>60 (63)	Total T <320 ng/dL	Testosterone enanthate injections	250 mg every three weeks	
Blackman 2002[11]	65-88 (72)	Total T <470 ng/dL	Testosterone enanthate injections	100 mg every two weeks	~600 ng/dL
Boyanov 2003[12]	68-89 (77.6)	Total T <435 ng/dL	Oral T undecanoate	120 mg daily	Not provided
Brockenbrough, 2006[13]	>18 (58.9)	Total T <300ng/dL	Transdermal 1% gel (Testim)	10 g gel	304 ng/dL
Cavallini 2004[14]	>60 (66)	Total T threshold not specified	Oral T undecanoate	160 mg daily	324 ng/dL
Clague 1999[15]	>60 (68.1)	Total T <401 ng/dL	Testosterone enanthate injections	200 mg every two weeks	559 ng/dL
Copenhagen Study group[16]	24-79 (53)	Not specified	Micronized oral testosterone	200 mg every 8 hours	Not provided
Crawford 2003[17]	>20 (58.7)	Not specified; baseline T <430 ng/dL	Mixed testosterone esters	200 mg every two weeks	~550 ng/dL
Drinka 1995[18]	60-90 (not reported)	Total T <320 ng/dL	Testosterone enanthate	150 mg/70 kg every two weeks	Not reported
Emmelot-Vonk 2008[19]	60-80 (67.1)	Total T <393 ng/dL	Oral T undecanoate	80 mg twice daily	Unchanged
English 2000[20]	Not specified (62.2)	T threshold not stated; baseline T in T-treated men ~388 ng/dL	Transdermal patch	5 mg daily	640 ng/dL
Ferrando 2003[21]	>60 (68)	Total T<480 ng/dL	Testosterone enanthate injections	100 mg weekly	659 ng/dL
Giannoulis 2006[22]	65-80 (70.3)	T threshold not specified; mean T level at baseline 499 ng/dL in the T group	Transdermal patch	5 mg daily	715 ng/dL
Hall 1996[23]	34-79 (60.8)	T levels not an inclusion criterion	T enanthate injections	250 mg monthly for 6 months followed by 250 mg every two weeks for an additional 3	892 ng/dL at end of treatment

				months	
Kenny 2004[24]	≥65 (80)	Bioavailable T <128 ng/dL	T enanthate injections	200 mg every three weeks	1211 ng/dL
Kenny 2001[25]	(76)	Bioavailable T<128 ng/dL	T transdermal patch	5 mg daily	636 ng/dL
Malkin 2006[26]	>18 (64)	No specified T threshold used as an inclusion criterion; baseline mean total T 401 and 349 ng/dL in T and placebo groups, respectively	Transdermal T patch	5 mg daily	562 ng/dL
Marks 2006[27]	44-78 (68)	Total T <300 ng/dL	T enanthate	150 mg every two weeks	640 ng/dL
Merza 2005[28]	>40 (63)	Total T <287 ng/dL	Transdermal T patch	5 mg daily	465 ng/dL
Morley 1993[29]	(77.6)	Bioavailable T <70 ng/dL	T enanthate injections	200 mg every two weeks	
Nair 2006[30]	>60 (66.2)	Bioavailable T<103 ng/dL ; baseline mean total T 371 ng/dL	Transdermal T patch	5 mg daily	483 ng/dL (421–651)
Page 2005[31]	>65 (71)	Total T <350 ng/dL	T enanthate injections	200 mg every two weeks	Nadir T ~600 ng/dL
Pugh 2004[32]	44-81 (median 62)	Not specified	Mixed T esters	100 mg every two weeks	Not provided
Reid 1996[33]	Not specified (61)	Not specified; baseline mean total T ~344 ng/dL	Mixed testosterone esters	250 mg monthly	877 ng/dL
Sattler 2009[34]	65-90 (70.7)	150-550 ng/dL	Testosterone transdermal gel	5 g or 10 g daily	523 ng/dL and 860 ng/dL on 5 and 10 g gel, respectivel y
Sih 1997[35]	≥50 (65)	Bioavailable T <60 ng/dL	T enanthate injections	200 mg every two weeks	461 ng/dL
Simon 2001[36]	No age range specified (53.1)	Total T <340 ng/dL	Testosterone transdermal gel	125 mg daily	Not provided
Snyder 1999[37]	>65 (73.1)	Total T <475 ng/dL	T scrotal transdermal patch	6 mg/day	625 ng/dL
Srinivas-Shankar 2010[38]	≥65 (73.7)	Total T <345 ng/dL or calculated free T <72 pg/mL	1% Testogel	Starting dose 50 mg gel; dose adjusted based on T levels	Mean 631 ng/dL at 6 months and 528 ng/dL at 3 months
Sullivan 2005[39]	≥65 (78.2)	Total T < 480ng/dL	T enanthate injections	100 mg weekly	804 ng/dL
Svartberg 2004[40]	54-74 (64.5)	Not specified	Testosterone enanthate	250 mg every month	~631 ng/L

Tan 2003[41]	Not specified (72)	Total T <240 ng/dL	Testosterone enanthate injections	200 mg every two weeks	341 ng/dL
Tenover 1992[42]	56-76 (67.5)	Total T <400 ng/dL	Testosterone enanthate injections	100 mg weekly	19.7 nmol/L
Wittert 2003[43]	≥60 (69)	Total T >229 ng/dL and free T index between 0.3 to 0.5	Oral T undecanoate	80 mg twice daily	No significant change in total T level

Legend: Testosterone thresholds used as the inclusion criterion, testosterone doses, and on-treatment serum testosterone levels reported in testosterone trials in middle-aged and older men. Trials that included men with classical hypogonadism were excluded. We also excluded trials in HIV-infected men because those men were young and had unique co-morbidities. Some trials did not report the age threshold for inclusion; in these trials, we listed the age range of included subjects. Some manuscripts did not report on-treatment testosterone values in the text or a table, but depicted them in a figure; in these cases, approximate values were inferred from the figure. In most studies that used injectable testosterone esters, the time of testosterone injection in relation to the blood draw was not specified. T, testosterone.

D. SUPPLEMENTARY APPENDIX FIGURES

Supplementary Appendix Figure 1. CONSORT diagram showing the flow of subjects through the study

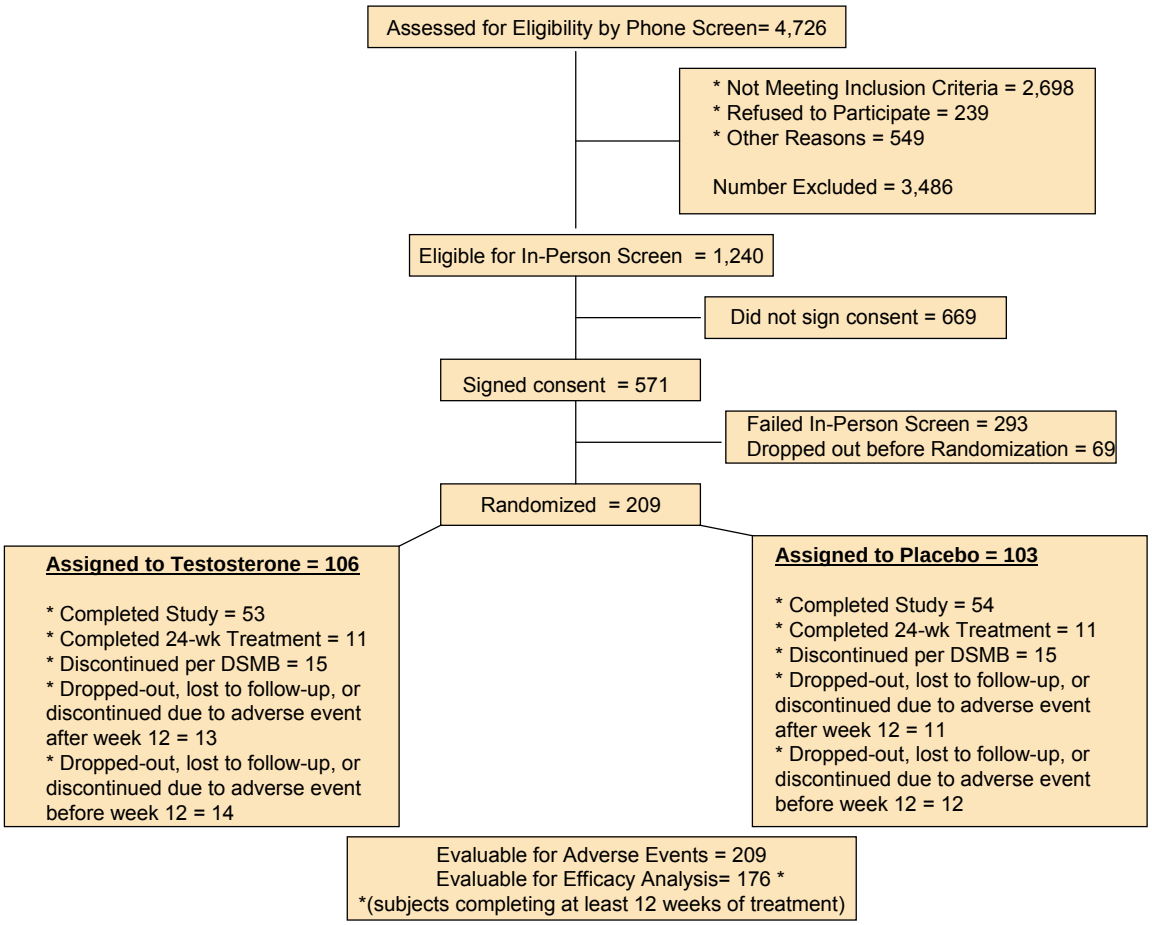


Figure Legend: We used a two stage screening process. In the first stage, after obtaining a verbal consent, conformity to a few key inclusion and exclusion criteria was determined during a structured telephone interview. Those who met these key inclusion and exclusion criteria were invited for the second stage screening when they signed a written consent form and underwent additional screening to determine eligibility.

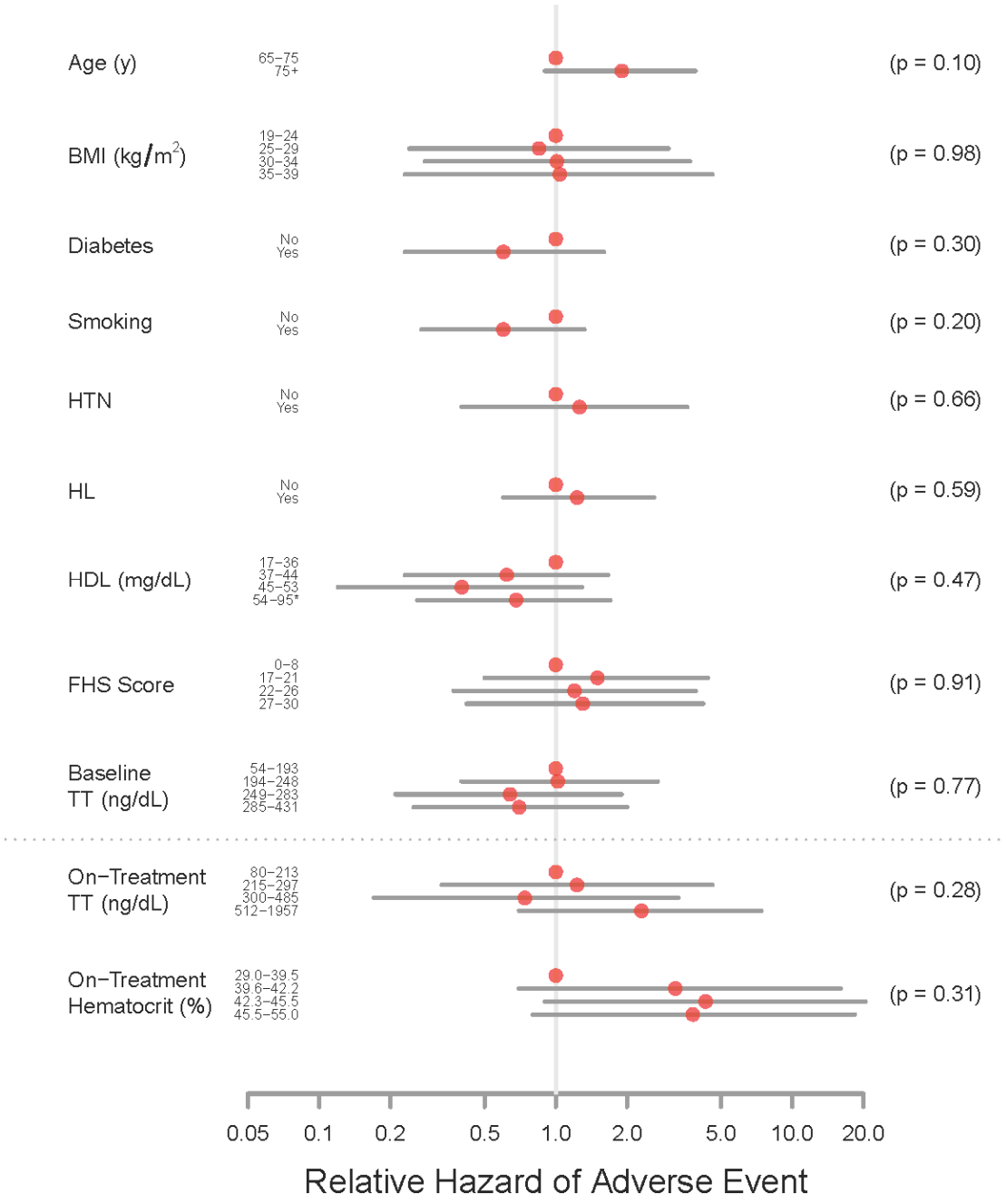
Amongst the 571 men who signed consent, 293 men failed in-person screening. The reasons for ineligibility in these 293 men were as follows: normal mobility or nonqualifying SPPB score (n=119), testosterone level outside the qualifying range (n=69), exclusionary medical conditions (n=31), exclusionary medications (n=8), PSA above the qualifying range (n=18), uncontrolled hypertension (n=7), high audit score (n=4), high AUA score (n=2), high BMI (n=2), high HbA1C (n=5), high hematocrit (n=4), high creatinine (n=1), language barrier (n=1), low MMSE score (n=1), unable to draw blood (n=2), and other (n=2). Seventeen subjects had more than one reason for exclusion.

*At the time when further enrollment was stopped and study medication was discontinued due to DSMB's recommendations, 209 men had been randomized as of December 15, 2009 and 107 (54 in testosterone and 53 in placebo group) had completed all phases of the study. An additional 22 men (11 in each group) had completed the full 24-weeks of study medication. Amongst those who did not complete the study either because they were lost to follow-up or discontinued because of DSMB's directive or for other reasons, 47 men (including 24 men (13 in testosterone and 11 in the placebo arm) who were lost to

follow-up or discontinued due to adverse events plus 23 men (15 in testosterone and 8 in placebo arm) who were discontinued per DSMB's directive had received at least 12-weeks of study medication and had undergone at least one post-randomization outcome assessment. These 176 men, who had completed baseline assessments and at least one post-randomization efficacy assessment, were eligible for analyses of efficacy data. All 209 randomized men were included in the adverse event analyses.

Of the 50 randomized subjects, who dropped out, were lost-to-follow up or discontinued, 27 (13 in the testosterone arm and 14 in the placebo arm) dropped out or were discontinued due to adverse events listed below. The adverse events reported by these 27 men included reaction to study gel (n=8), sleep disturbances (n=3), cardiovascular-related event (n=4), prostate-related (n=3), medical co-morbidities (n=3), high hematocrit (n=1), musculoskeletal (n=1), gastrointestinal bleeding (n=1), neuropathy (n=1), asthma exacerbation (n=1), and lung cancer (n=1).

Supplementary Appendix Figure 2



Legend: Estimated hazard ratios for the occurrence of a cardiovascular-related adverse event are presented with respect to risk factor categories, along with 95% confidence intervals. Estimates were obtained using Cox proportional hazards modeling. Reference categories are presented along the line of no effect (1.0; gray) and without confidence intervals. At bottom are presented estimates for total testosterone (TT) and hematocrit categories at the end of the treatment phase; all other estimates represent baseline risk factor values. BMI, body mass index; FHS, Framingham Heart Disease; HTN, hypertension; HL, hyperlipidemia

E. SUPPLEMENTARY APPENDIX REFRENCES

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