

Effect of Testosterone Replacement Therapy on Sexual Function and Hypogonadal Symptoms in Men with Hypogonadism

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Abstract

Background. Few long-term randomized trials have evaluated the efficacy of testosterone replacement therapy (**TRT**) in improving sexual function and hypogonadal symptoms in men with hypogonadism and whether effects are sustained beyond 12 months.

Objective. Testosterone Replacement therapy for Assessment of long-term Vascular Events and efficacy ResponSE in hypogonadal men (TRAVERSE) study evaluated the effect of TRT on major adverse cardiovascular events in middle-aged and older men with hypogonadism. The Sexual Function Study, nested within the parent trial, determined testosterone's efficacy in improving sexual activity, hypogonadal symptoms, libido, and erectile function among men reporting low libido.

Methods. Among 5204 men, 45 to 80 years, with two testosterone concentrations <300 ng/dL, hypogonadal symptoms, and cardiovascular disease (CVD) or increased CVD risk enrolled in TRAVERSE trial, 1161 with low libido were enrolled in the Sexual Function Study (587 randomized to receive 1.62% testosterone gel and 574 to placebo gel for the duration of their participation in the study). Primary outcome was change from baseline in sexual activity score. Secondary outcomes included hypogonadal symptoms, erectile function, and sexual desire.

Results. TRT was associated with significantly greater improvement in sexual activity than placebo [estimated mean (95% confidence interval) between-group difference 0.49 (0.19,0.79) and 0.47 (0.11,0.83) acts per day] at 6 and 12 months, respectively, omnibus test $p=0.011$]; treatment effect was maintained at 24 months. TRT improved

1 hypogonadal symptoms and sexual desire, but not erectile function, compared to
2 placebo.

3 **Conclusions:** In middle-aged and older men with hypogonadism and low libido, TRT
4 for two years improved sexual activity, hypogonadal symptoms, and sexual desire, but
5 not erectile function.

6 **Trial Registration: ClinicalTrials.gov Identifier: NCT03518034**

INTRODUCTION

Sexual symptoms are common in middle-aged and older men (1-3) and the most frequent reason that motivates middle-aged and older men to seek testosterone treatment (4,5). Sexual desire, erectile function and overall sexual activity decline with advancing age as do total and free testosterone levels (1,6,7). Low total and free testosterone levels are associated with low libido and erectile dysfunction in middle-aged and older men (8-11).

Testosterone has been shown to stimulate sexual thoughts and fantasies, attentiveness to erotic cues, spontaneous and night-time erections, penile blood flow and veno-occlusion (12-16). However, randomized trials data on the efficacy of testosterone replacement therapy (TRT) in improving sexual function and hypogonadal symptoms in middle-aged and older men with hypogonadism are sparse. Most trials of TRT have been open-label studies (13,14,17-21). Only a few placebo-controlled, randomized trial have evaluated the efficacy of TRT in improving sexual function in men with hypogonadism using validated assessment methods (22-24). Among these, two trials (23,24) were of 3-month duration and only one trial — the Testosterone Trials (TTrials) — was 12 months in duration (22). However, no randomized trial has evaluated whether the effects of TRT on various domains of sexual function are sustained beyond 12 months in men with hypogonadism. The TTrials did not include middle-aged men less than 65 years of age with hypogonadism, who constitute the largest segment of men receiving testosterone therapy (25,26). No previous trial has evaluated whether TRT improves hypogonadal symptoms, using a validated symptom questionnaire.

1 The Testosterone Replacement therapy for Assessment of long-term Vascular
2 Events and efficacy ResponSE in hypogonadal men (TRAVERSE) Study was designed
3 to evaluate the effect of TRT and placebo on major adverse cardiovascular events
4 (MACE) in men with hypogonadism with or at increased risk of cardiovascular disease
5 (CVD) (27). To enable a parallel assessment of potential benefits of TRT, the
6 TRAVERSE Sexual Function Study, nested within the main trial, evaluated the efficacy
7 of TRT in improving sexual activity, using an integrated measure of sexual function, and
8 other validated efficacy endpoints used previously in TRT trials in middle-aged and
9 older men with hypogonadism (20,22-24). The study determined whether TRT improves
10 symptoms of hypogonadism, ascertained using a validated instrument (5), and the
11 durability of treatment effect beyond the first year of intervention. The effects of TRT on
12 erectile function, sexual desire, and sexual symptoms were evaluated over two years.

MATERIALS AND METHODS

The design and the primary MACE results of the TRAVERSE trial have been published (27,28). Briefly, the TRAVERSE trial was a randomized, double-blind, placebo-controlled, parallel group study conducted at 316 trial sites in the United States. The participants were men, 45 to 80 years of age, who had two fasting, morning serum testosterone concentrations drawn at least 48 hours apart <300 ng/dL and one or more signs or symptoms of hypogonadism, and either pre-existing cardiovascular disease (CVD) or increased risk of CVD (27,28). Men who had a contraindication for testosterone treatment (e.g., erythrocytosis, history of prostate cancer, prostate nodule, severe lower urinary tract symptoms indicated by International Prostate Symptom Score >19 , or PSA >3 ng/mL or >1.5 ng/mL if using steroid 5 α -reductase inhibitor) were excluded. The participants in the Sexual Function Study had to meet the eligibility criteria for the parent trial; in addition, they had to have low libido, as indicated by DeRogatis Interview for Sexual Functioning - Desire score < 20 because TRT has been shown to improve sexual function consistently only in men with hypogonadism who have low libido (22,24). Participants who did not complete the PDQ-4 instrument on at least 4 days during the baseline period were excluded.

The participants were randomized with stratification for pre-existing CVD to receive, daily until study completion, either 1.62% transdermal testosterone gel (AndroGel, AbbVie, Inc. North Chicago, IL) or a matching placebo gel provided in metered dose pumps. The treatment allocation was double-blind; the participants, study investigators, and outcome assessors were unaware of the intervention assignment. The testosterone dose was adjusted centrally to maintain serum testosterone levels

1 between 350 and 750 ng/dL and hematocrit below 54%, guided by the on-treatment
2 testosterone levels and hematocrit levels, using a pre-specified algorithm that has been
3 described previously (27,28). Sham adjustments were made in participants in the
4 placebo group to maintain blinding.

5 The study protocol was approved by the national and local institutional review
6 boards for human subjects' research. An independent Data and Safety Monitoring
7 Committee reviewed study's progress and safety data every 6 months. All participants
8 provided written informed consent for participating in the parent trial and provided
9 additional consent to participate in the Sexual Function Study.

10 The study was funded by a consortium of testosterone manufacturers led by
11 AbbVie, Inc (North Chicago, IL). The Sexual Function Study's design and analyses
12 plans were crafted by the TRAVERSE Sexual Function Study Committee led by the
13 Research Program in Men's Health at the Brigham and Women's Hospital, Boston, MA
14 (Appendix).

15 **Study outcomes**

16 The primary outcome of the TRAVERSE Sexual Function Study, was the change
17 from baseline in sexual activity, assessed using the Psychosexual Daily Diary Question
18 4 (**PDQ-4**) (29). Participants were asked to complete PDQ-4 using a tablet device for 7
19 consecutive days prior to baseline and follow-up visits during months 6, 12 and 24. The
20 PDQ-4 question asks the participants to mark one or more sexual activities they
21 engaged in during the preceding week; the PDQ-4 score can range from 0 to 12, with
22 higher scores indicating greater sexual activity. Skipped questions were coded as 0 if at

1 least one of the 12 questions was nonmissing; final score for a visit was the average of
2 4-7 daily scores.

3 Secondary endpoints included change from baseline in symptoms of
4 hypogonadism, assessed using a validated, psychometrically robust instrument to
5 evaluate hypogonadal symptoms (5) that includes 28 questions addressing 5 domains
6 (sexual, energy, sleep, cognition, and mood) and 2 sexual subdomains (libido and
7 sexual symptoms). A higher HIS-Q composite score or domain/subdomain score
8 indicates worse status. The HIS-Q questionnaire was administered to all randomized
9 study participants while all other questionnaires were administered only to the
10 participants in the Sexual Function Study.

11 Erectile function was assessed by the International Index of Erectile Function
12 (IIEF-5) score (30), administered at baseline and during months 12 and 24. Participants
13 responded to questions over the past 6 months, with each item having responses from
14 1 (worst) to 5 (best). To calculate a total score, the values were summed if all five
15 responses were non-missing; if four were non-missing, the sum was normalized to five
16 (maximum score 25, with a higher score representing better function). If two or more
17 responses were missing the total score was set to missing.

18 The Patient Global Impression of Improvement (PGI-I) Libido question asked
19 participants to assess their libido at a follow-up visit in comparison with the start of the
20 study. Possible responses on a 7-point Likert scale ranged from "very much improved"
21 (value 0) to "very much worse" (value 6). Values less than 3 indicate improvement and
22 values greater than 3 indicate worsening.

Serum testosterone, dihydrotestosterone, and estradiol concentrations were measured using liquid chromatography tandem mass spectrometry (LC-MS/MS) assays in fasting, morning samples in a central laboratory (LabCorp, Inc, Indianapolis, IN) that is certified by the Hormone Standardization Program for Testosterone of the Center for Disease Control and Prevention. The lower limit of quantitation (LLOQ) for the testosterone assay was 2 ng/dL; inter-assay coefficients of variation (CV) at 2, 43, 194, and 913 ng/dL were 2.4%, 1.3%, 1.4%, and 1.7%, and the intra-assay CV at the corresponding concentrations were 8.6%, 5.6, 5.4, and 5.1%, respectively. For the estradiol LC-MS/MS assay, the LLOQ was 0.5 pg/mL, the inter-assay and intra-assay CVs in quality control pools with estradiol concentrations at the LLOQ, low, medium and high range were 7.3%, 7.5%, 5/8%, 8.1% were: 14.1%, 2.5%, 1.8%, and 2.0%, respectively. For the dihydrotestosterone (DHT) assay, the LLOQ was 10 ng/dL, and the inter- and intra-assay CVs in quality control pools with DHT concentrations in the low, medium and high male range were 3.1%, 3.0%, and 1.6%, and 3.0%, 2.9%, and 2.6% respectively.

Statistical Analyses

The analyses were performed on the intent-to-treat analytic cohort. The primary outcome, the change from baseline in PDQ-4 score, was analyzed using linear mixed regression model with fixed effects for treatment, visit, treatment-by-visit interaction, and adjusted for baseline PDQ-4 value and pre-existing CVD. All randomized participants enrolled in the Sexual Function study with available questionnaire data at each time point were analyzed; these numbers at each time point are shown in the figures and tables. The random per-subject repeated measure effect was included in the model. An

unstructured covariance matrix was assumed unless otherwise noted. The least squares mean of the effect difference between TRT and placebo groups accompanied by 95% confidence intervals (**CI**) are presented for each timepoint. An omnibus F-test was used to evaluate whether there was a significant difference between testosterone and placebo groups across all study. Secondary analyses of difference between TRT and placebo in changes from baseline in composite HIS-Q score (hypogonadal symptoms), sexual function (sexual symptoms) domain, and libido subdomains, as well as hormone levels, were conducted in a similar fashion as the primary outcome using mixed regression model. Pre-specified efficacy analyses were performed in subgroups of men categorized by baseline testosterone (<250 , ≥ 250 ng/dL), pre-existing CVD (yes, no), race (Black or African-American, White), and age (<65 , 65 or older). Post-hoc sensitivity analysis of the primary outcome excluding men who were using a phosphodiesterase 5 inhibitor, as well as analyses of average change from baseline in erectile function in testosterone-treated men with an average on-treatment testosterone level > 450 ng/dL versus testosterone level ≤ 450 ng/dL were also conducted.

As described previously (27,28), the study was projected to enroll 6,000 participants and accrual of at least 256 composite MACE events to test if testosterone intervention is non-inferior to placebo with 95% (2-sided) upper confidence limit of the hazard ratio less than 1.5 for the estimated MACE incidence with 90% statistical power. The enrollment was stopped on February 1, 2022, based upon blinded assessments of pooled accrual rates of MACE (28).

Hypothesis testing utilized two-sided significance alpha level of 0.05. No adjustment was made for multiplicity. The analyses were performed using SAS Version

9.4 (SAS Institute, Cary, NC) and R version 4.2.1 (R Foundation for Statistician Computing, Vienna).

RESULTS

The first participant was enrolled on May 23, 2018, and enrollment was stopped on February 1, 2022. The final study assessments were completed on January 19, 2023. After excluding 42 identification numbers with duplicate enrollment, the Full Analysis Set (FAS) included 5204 randomized participants, 2601 in TRT group and 2603 in placebo group; among these, 1161 participants, who consented to participate in the Sexual Function Study, met the eligibility criteria for the Sexual Function Study, and had baseline PDQ-4 data were enrolled in Sexual Function Study, 587 in the TRT and 574 in the placebo group.

The baseline characteristics of the participants in the TRT and placebo groups in the Sexual Function Study were similar (**Table 1**). The mean (standard deviation; SD) age of the participants in the Sexual Function Study was 63.7 (7.6) years; 578 (49.8%) were 65 years or older. The mean PDQ-4 score was 1.8 (1.7), IIEF score 12.9 (5.7) and HIS-Q Composite Score of 46.9 (11.7). Among men enrolled in the Sexual Function Study, 8.7% and 9.8% of men in the TRT and placebo groups, respectively, reported using a selective phosphodiesterase 5 inhibitor at baseline (**Table 1**). A similar proportion of the men in the testosterone (11.7%) and placebo (9.9%) groups in the Sexual Function Study were not sexually active at baseline.

Increases in serum testosterone, DHT, and estradiol levels were significantly greater in testosterone-treated men than placebo-treated men in Sexual Function Study (Table 2).

Primary Endpoint: Sexual Activity. The participants in the TRT group had a significantly greater increase in their average daily sexual activity than men in the placebo group at months 6 and 12 (estimated between-group difference 0.49, 95% CI: 0.19, 0.79, 0.47, 95% CI: 0.11, 0.83, and 0.48, 05% CI: -0.01, 0.96, at 6, 12 and 24 months, respectively; omnibus test $p=0.011$; **Figure 1**). The treatment effect on sexual activity at 24 months did not differ from that at 12 months (F-test $p = 0.978$).

Secondary Endpoints

Among men enrolled in the Sexual Function Study, the TRT group experienced a significantly greater improvement in hypogonadal symptoms than the placebo group at all time points, indicated by a significantly larger decrease in HIS-Q composite score (lower symptom score indicates fewer symptoms; estimated between-group difference, -2.3, 95% CI: -3.6, -1.0 at 6 months; -2.5, 95% CI: -3.9, -1.1 at 12 months; and -2.7, 95% CI: -4.3, -1.03 at 24 months, respectively, omnibus test $p = 0.001$; **Figure 2**).

The improvement in sexual symptoms assessed using the HIS-Q sexual function domain in men enrolled in the Sexual Function Study, was significantly greater in the TRT group than in placebo group (estimated between-group differences -2.7, 95% CI: -5.1, -0.3 at 6 months; -4.0, 95% CI: -6.8, -1.3 at 12 months; and -3.4, 95% CI: -6.4, -0.3 at 24 months, respectively; omnibus test $p = 0.019$). Sexual desire, assessed using the libido subdomain of HIS-Q, improved significantly more in the TRT than in the placebo group (estimated between-group differences: -3.9, 95% CI: -5.8, -1.9 at 6 months; -3.3,

95% CI: -5.5, -1.1 at 12 months; and -3.4, 95% CI: -5.9, -0.9 at 24 months, respectively, omnibus test $p=0.001$; **Figure 2**).

When the participants were asked to assess their libido at a follow-up visit in comparison with the start of the study using the Patient Global Impression of Improvement Libido question, testosterone-treated men reported greater improvement in their libido than placebo-treated men [estimated between-group difference -0.4 [-0.5, -0.2] and -0.3 [-0.5, -0.2] at 12 and 24 months, respectively, omnibus test $p<0.001$ (**Figure 3**).

Changes in erectile function, assessed using the IIEF-5 did not differ significantly between the TRT and the placebo groups (estimated between-group differences: 0.6, 95% CI: -0.4, 1.6 at 12 months, and -0.2, 95% CI: -1.7, 1.4, at 24 months, omnibus test $p=0.443$; **Figure 4**). A post-hoc sensitivity analysis excluding men who were using a phosphodiesterase 5 inhibitor at baseline yielded similar results (estimated between-group differences: 0.4, 95% CI: -0.7, 1.5 at 12 months, and 0.2, 95% CI: -1.6, 1.9 at 24 months, omnibus test $p=0.752$). A post-hoc analyses did not find any difference in the average change from baseline in erectile function between testosterone-treated men with an average on-treatment testosterone level ≥ 450 ng/dL versus testosterone level ≤ 450 ng/dL ($p=0.178$).

Among all randomized TRAVERSE participants, hypogonadal symptoms also improved more in testosterone-treated than in placebo-treated men [estimated between-group difference in the HIS-Q composite score: -1.6 (-2.2, -0.9) at 6 months; -1.7 (-2.3, -1.0) at 12 months; and -1.5 (-2.3, -0.7) at 24 months; omnibus test $p<0.001$] (**Figure 5**,

Panel A). Similarly, among all randomized TRAVERSE participants, the overall sexual symptoms domain score ($p=0.001$) and sexual desire score ($p<0.001$) improved more in the TRT group than in placebo group (**Figure 5, Panels B and C**).

Pre-specified Subgroup Analyses

The treatment effect appeared to be greater in men ≥ 65 years of age than in those < 65 years, and in men with prior CVD than in those without CVD, but the interaction test did not show statistically significant difference between the subgroups by age ($p=0.338$) or by prior CVD ($p=0.421$). The p values for the interaction test for the primary endpoint stratified by baseline testosterone level ($< 250, \geq 250$ ng/dL) and race (White or Black American) were also not statistically significant (see Figure 6 Legend).

DISCUSSION

The TRAVERSE Sexual Function Study, the largest randomized, placebo-controlled study of the effects of TRT on sexual function, demonstrated the efficacy of TRT relative to placebo in improving overall sexual activity during 12 months of treatment. These results are similar to the TTrials in men, 65 years or older, with hypogonadism (22). The study also demonstrated the durability of the treatment effect at 24 months that was similar to that at 12 months. The study provided evidence of the efficacy of TRT in improving the symptoms of hypogonadism using a validated instrument. In addition, the testosterone-treated men reported greater improvements in their sexual symptoms and sexual desire. Because a majority of middle-aged and older men who seek testosterone treatment do so because of sexual symptoms, and sexual symptoms are the most common manifestation of hypogonadism, these findings will be

1 helpful to men with hypogonadism and their clinicians in guiding their decision to initiate
2 or continue TRT.

3 Sexual activity is a composite marker of sexual function; testosterone-treated
4 men increased their frequency of daily sexual activity by an average of 0.97 events or
5 6.8 events per week which represents nearly a 50% increase in their overall sexual
6 activity. The men in the placebo group also reported an increase in their frequency of
7 sexual activity but the frequency of sexual activity increased more in the testosterone-
8 treated than placebo-treated men by an average of 0.47 events per day or 3.3 events
9 per week (approximately a 25% increase relative to placebo). Several considerations
10 can help place in perspective the magnitude of the observed improvements in sexual
11 activity and desire. First, the patient's perceptions of whether these improvements in
12 sexual activity and desire were beneficial may be influenced by many factors, including
13 age, partner availability, sexual beliefs and practices, baseline sexual activity, mood,
14 and the burden of co-morbid conditions. By virtue of the eligibility criteria, the study
15 population had high burden of chronic conditions such as CVD, diabetes, hypertension,
16 and obesity. Second, even though a majority of older men continue to be sexually
17 active, sexual activity decreases as men grow older (1,3). An observational study found
18 the average frequency of partnered sexual activity in men to decrease from 7.2 per
19 month in men 30 to 39 years of age to nearly half in men, aged 80 years (31-33). In
20 another study of US adults aged 57 to 85 years, nearly three fourths were sexually
21 active and approximately 50% of those who were sexually active reported engaging in
22 partnered sexual activity 2 or 3 times a month (1). The TRAVERSE Sexual Function
23 study did not have a requirement for partner availability or prior sexual activity and a

substantial fraction (nearly 10.8%) was not sexually active at baseline. Furthermore, PDQ-4 score includes nonpartnered activities such as masturbation; therefore, the event rates derived from PDQ-4 in this study cannot be directly compared to the data from prior observational studies. Even so, an absolute mean increase of approximately 50% above baseline and a relative 25% greater increase over placebo treatment appears favorable in the context of these observational data of sexual activity in middle-aged and older adults. Third, the findings of a significantly greater improvement in sexual desire assessed using the HIS-Q sexual desire subdomain score in the TRT than placebo group was corroborated by the corresponding greater improvement in the patient global impression of change in sexual desire in the TRT versus placebo group.

Meta-analyses of the testosterone trials have reported increases in sexual desire and variable changes in erectile function in men with low testosterone levels (19,34). However, most testosterone trials were not randomized and did not include a placebo control, and they enrolled relatively small number of men; many trials enrolled men without symptoms of hypogonadism or men with low normal testosterone levels, and only a few used validated instruments to measure sexual activity, sexual desire, and erectile function. In an individual patient data meta-analysis, the erectile function did not improve with TRT in studies that used IIEF-5 (35). Even in the few trials that reported improvements in erectile function (22,36), the magnitude of the improvement in erectile function was small. It is possible that high prevalence of CVD and diabetes among the TRAVERSE participants, similar to that in men who are receiving TRT in real world practice, may have attenuated the erectile response to TRT. The treatment effect on sexual activity was slightly smaller than that in the TTrials. However, the study population

1 enrolled in the TRAVERSE Trial differed from that in the TTrial in several ways. By virtue of the
2 eligibility criteria, the TRAVERSE participants had a substantially higher rates of CVD, diabetes
3 and other CVD risk factors than the TTrial participants. Also, the TTrial required participants
4 to have a sexual partner and be sexually active at screening. These baseline differences in the
5 study populations could have differentially influenced the treatment response in the two trials.

6 The Testosterone Effects on Atherosclerosis Progression in Aging Men (The TEAAM
7 Trial) did not find any improvement in any domain of sexual function in middle-aged and older
8 men with low or low normal testosterone levels treated with placebo or testosterone gel for 3
9 years (37); however, the men enrolled in the TEAAM trial were not required to have sexual
10 symptoms and did not meet the definition of hypogonadism.

11 Several aspects of the trial are notable. The trial enrolled men, who had two
12 fasting morning testosterone levels < 300 ng/dL as well as one or more symptoms of
13 hypogonadism, consistent with the recommendations of the Endocrine Society's
14 guideline (38). The primary and secondary endpoints were ascertained for up to 2 years
15 longer than any previous randomized trial in men with hypogonadism. Psychometrically
16 robust, validated instruments were used to evaluate sexual activity, sexual desire,
17 erectile function, and hypogonadal symptoms. Because the trial enrolled men with pre-
18 existing CVD or 3 or more risk factors for CVD, it is not surprising that the participants
19 had high prevalence of diabetes, obesity and CVD that are risk factors for sexual
20 dysfunction. Surveys of men with hypogonadism or receiving TRT in real world
21 practices also have revealed high rates of diabetes, obesity and CVD.

22 The trial has some limitations. The participants were not required to have a
23 sexual partner and some participants were not sexually active at baseline, not dissimilar

1 from the general population of community dwelling older adults (6). As TRAVERSE was
2 an event-driven trial and the participants were followed until the accrual of the projected
3 number of MACE, some participants did not complete 2 years of treatment. The non-
4 retention rates in the Sexual Function Study were high but similar in the TRT and
5 placebo groups. Similar nonretention levels have been reported in other long-term trials
6 of testosterone (37,39), in real world clinical practice (40), and in other treatment trials
7 for symptomatic conditions, such as chronic pain (41) and menopause (42). The
8 nationwide shutdown during the SARS-CoV-2 pandemic prevented some participants
9 from coming to the trial sites for completing the questionnaires. However, the
10 nonretention would only bias the results towards null making the significant findings of
11 improvements in sexual activity, sexual desire, and hypogonadal symptoms over one
12 year and the durability of the treatment effect at 2 years all the more notable. Sexual
13 activity and sexual desire also increased in the placebo group; similar placebo effects have
14 been reported in other symptomatic conditions, such as pain disorders, depression, as well as in
15 men and women with sexual disorders (43-45). However, the testosterone treatment was
16 associated with significantly greater improvements in sexual activity, sexual desire, and
17 hypogonadal symptoms than placebo treatment.

18 Sexual activity is an important marker of mental and physical well-being and
19 quality of life, in middle-aged and older adults (46). Therefore, the findings of the trial
20 will facilitate a more informed consideration of the potential benefits and risks of TRT by
21 middle-aged and older men with hypogonadism and their clinicians.

22 **Conclusions.** Testosterone treatment of middle-aged and older men with
23 hypogonadism who had low sexual desire was associated with significantly greater

improvements in overall sexual activity and sexual desire but not erectile function. Testosterone treatment improved hypogonadal symptoms, including sexual symptoms, more than placebo. The improvements in sexual activity, sexual desire, and hypogonadal symptoms were maintained for up to two years of treatment.

Acknowledgements

The TRAVERSE Sexual Function Study Committee

The TRAVERSE Sexual Function Study Subcommittee of the TRAVERSE Study crafted the protocol and Statistical Analyses Plan for the analyses of the sexual function study data; performed the analyses of the Sexual Function Study data, and was led by Shalender Bhasin, MB, BS, at the Harvard Medical School, Brigham and Women's Hospital in Boston, MA. The Subcommittee's members included Shalender Bhasin, MB, BS (Chair), Harvard Medical School, Brigham and Women's Hospital, Boston, MA; TX; Glenn R. Cunningham, MD, Baylor College of Medicine, Houston, TX; Thomas G. Travison, PhD, Marcus Institute of Aging Research, Boston, MA; Karol M. Pencina, PhD, Harvard Medical School, Brigham and Women's Hospital in Boston, MA; Kathleen Wannemuehler, PhD, University of Wisconsin, Madison, WI; Lauren Wilson, RNP, Brigham and Women's Hospital, Boston, MA; Neha Rupeja, Brigham and Women's Hospital, Boston, MA; Thiago Galgiano, MD, PhD, Brigham and Women's Hospital, Boston, MA.

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Trial Sites

We are grateful for the important contributions of the staff and investigators of the trial sites to the successful completion of the trial. A list of trial sites has been published in Lincoff et al (reference 28). We also thank the trial participants for their partnership in this trial even in the midst of the COVID pandemic.

Data Availability Statement

The de-identified data presented in this manuscript will be made available for research purpose only upon submission of a request and its review by the TRAVERSE Sexual Function Study Committee. The requests can be submitted by email to Dr. Shalender Bhasin (sbhasin@bwh.harvard.edu).

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1 Table 1 Baseline Characteristics of Participants in the TRAVERSE Sexual Function
2 Trial and in the Full Analysis Set

| | TRAVERSE Full Analysis Set | | TRAVERSE Sexual Function Study | |
|---|----------------------------|--------------------------|--------------------------------|-------------------------|
| Variable | TRT (N = 2601) | Placebo (N = 2603) | TRT (N = 587) | Placebo (N = 574) |
| Age | 63.3 (7.9) | 63.3 (7.9) | 64.0 (7.5) | 63.3 (7.8) |
| Age Group | | | | |
| 45 - <65 Yrs | 1360 (52.3%) | 1392 (53.5%) | 288 (49.1%) | 295 (51.4%) |
| 65+ Yrs | 1241 (47.7%) | 1211 (46.5%) | 299 (50.9%) | 279 (48.6%) |
| Race Group | | | | |
| White | 2070 (79.6%) | 2084 (80.1%) | 488 (83.1%) | 475 (82.8%) |
| Black/ African Am. | 445 (17.1%) | 432 (16.6%) | 82 (14.0%) | 75 (13.1%) |
| Other | 86 (3.3%) | 87 (3.3%) | 17 (2.9%) | 24 (4.2%) |
| Ethnicity | | | | |
| Hispanic / Latino | 409 (15.7%) | 439 (16.9%) | 56 (9.5%) | 70 (12.2%) |
| Not Hispanic / Latino | 2191 (84.3%) | 2162 (83.1%) | 531 (90.5%) | 504 (87.8%) |
| Missing | 1 (-) | 2 (-) | | |
| Prior CVD | 1410 (54.2%) | 1437 (55.2%) | 289 (49.2%) | 294 (51.2%) |
| At Risk for CVD | 1191 (45.8%) | 1166 (44.8%) | 298 (50.8%) | 280 (48.8%) |
| Prior Testosterone Use | 5 (0.2%) | 10 (0.4%) | 2 (0.3%) | 2 (0.3%) |
| Dyslipidemia | 2344 (90.1%) | 2332 (89.6%) | 542 (92.3%) | 527 (91.8%) |
| Hypertension | 2423 (93.2%) | 2402 (92.3%) | 559 (95.2%) | 529 (92.2%) |
| Nicotine use | 527 (20.3%) | 534 (20.5%) | 113 (19.3%) | 121 (21.1%) |
| Testosterone (ng/dL) | 220.5 (47.0) [n=2596] | 220.1 (48.1) [n=2602] | 220.6 (46.2) | 219.2 (47.5) |
| Dihydrotestosterone (ng/dL) | 16.1 (7.9) [n = 2487] | 16.2 (8.5) [n = 2498] | 15.8 (8.0) [n = 571] | 15.8 (7.3) [n = 557] |
| Estradiol (pg/mL) | 21.0 (8.2) [n = 2470] | 21.0 (8.4) [n = 2494] | 20.9 (8.3) [n = 569] | 21.0 (7.6) [n = 551] |
| Sexual Activity PDQ-4 Score per day | 1.8 (1.7) [n = 631] | 1.9 (1.8) [n = 617] | 1.8 (1.7) | 1.8 (1.8) |
| Number (%) not sexually active at baseline (PDQ-4 Score = 0) | 74 (11.7%) [n = 631] | 61 (9.9%) [n = 617] | 70 (11.9%) | 59 (10.3%) |
| HIS-Q Total Score | 46.1 (12.0) [n = 2402] | 45.8 (12.0) [n = 2381] | 46.9 (11.5) [n = 563] | 46.8 (12.0) [n = 550] |
| HIS-Q Libido Subdomain Score | 59.0 (18.2) [n = 2420] | 59.2 (18.3) [n = 2398] | 65.4 (16.8) [n = 567] | 65.8 (17.1) [n = 553] |
| HIS-Q Sexual Function Subdomain Score | 61.3 (20.1) [n = 2415] | 60.6 (20.6) [n = 2396] | 63.1 (19.0) [n = 565] | 63.4 (19.7) [n = 553] |
| Baseline DISF Desire Score | 14.7 (7.3) [n = 2581] | 14.6 (7.4) [n = 2587] | 12.0 (5.3) | 12.0 (5.2) |
| Baseline IIEF Total Score | 12.9 (5.5) [n = 708] | 12.8 (5.5) [n = 704] | 13.0 (5.7) [n = 328] | 12.8 (5.7) [n = 325] |
| Using a PDE5 inhibitor | 227 (8.7%) | 251 (9.6%) | 51 (8.7%) | 56 (9.8%) |

3

4 **Legend.** The data are mean (standard deviation, SD) unless otherwise specified. If the
5 total number of available records was different than total population set, the actual
6 sample size is presented in the brackets. To convert serum total testosterone
7 concentrations in nanograms per deciliter to nanomoles per liter, multiply testosterone

1 concentration in nanograms per deciliter by 0.0347. To convert estradiol concentrations
2 from picogram per milliliter to picomoles per liter, multiply estradiol concentrations in
3 picogram per milliliter by 3.67. To convert dihydrotestosterone concentrations in
4 nanograms per deciliter to nanomoles per liter, multiply dihydrotestosterone
5 concentrations in nanograms per deciliter by 0.0344.

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1 Table 2. Post-randomization Testosterone, DHT and Estradiol

2

| Test | Month | Study Arm | N | Baseline Mean (SD) | Visit Mean (SD) | Change from baseline | | Treatment Difference | | P |
|----------------------|-------|-----------|-----|--------------------|-----------------|----------------------|----------------|----------------------|----------------|-------|
| | | | | | | LS Mean | 95% CI | LS Mean | 95% CI | |
| Testosterone (pg/mL) | 0.5 | TRT | 552 | 220.6 (46.2) | 370.2 (184.0) | 149.8 | (138.4,161.1) | 142.5 | (126.2,158.7) | <.001 |
| | | Placebo | 532 | 219.2 (47.5) | 227.1 (72.1) | 7.3 | (-4.3, 18.9) | | | |
| | 1 | TRT | 572 | 220.8 (46.3) | 417.5 (284.6) | 197.2 | (180.3, 214.2) | 188.7 | (164.5, 212.9) | |
| | | Placebo | 555 | 219.0 (47.1) | 228.1 (69.9) | 8.5 | (-8.7, 25.8) | | | |
| | 3 | TRT | 551 | 221.5 (46.4) | 444.3 (434.3) | 223.4 | (197.0, 249.7) | 213.1 | (175.5, 250.7) | |
| | | Placebo | 532 | 219.2 (47.6) | 229.8 (93.4) | 10.3 | (-16.5, 37.1) | | | |
| | 6 | TRT | 508 | 220.2 (46.6) | 446.0 (292.8) | 226.1 | (207.4, 244.8) | 212.4 | (185.8, 239.1) | |
| | | Placebo | 491 | 220.2 (46.9) | 234.8 (86.1) | 13.7 | (-5.3, 32.7) | | | |
| | 12 | TRT | 404 | 220.3 (46.4) | 477.1 (399.2) | 253.7 | (225.5, 282.0) | 230.2 | (190.4, 270.0) | |
| | | Placebo | 412 | 218.4 (48.2) | 242.6 (111.7) | 23.5 | (-4.5, 51.5) | | | |
| | 18 | TRT | 360 | 221.5 (46.4) | 448.6 (321.9) | 227.0 | (203.1, 250.9) | 204.6 | (170.4, 238.8) | |
| | | Placebo | 345 | 217.3 (48.7) | 242.2 (98.9) | 22.4 | (-2.0, 46.8) | | | |
| | 24 | TRT | 309 | 221.3 (45.5) | 414.7 (274.3) | 195.2 | (170.6, 219.7) | 155.6 | (120.4, 190.7) | |
| | | Placebo | 292 | 217.5 (49.1) | 258.3 (162.3) | 39.6 | (14.4, 64.8) | | | |
| | 36 | TRT | 222 | 222.0 (46.0) | 406.8 (275.0) | 187.3 | (159.3, 215.3) | 142.1 | (102.7, 181.5) | |
| | | Placebo | 227 | 218.2 (47.0) | 264.9 (144.0) | 45.2 | (17.5, 72.9) | | | |
| | 48 | TRT | 77 | 223.1 (46.0) | 322.3 (169.9) | 108.4 | (79.0,137.8) | 81.4 | (37.4, 125.4) | |
| | | Placebo | 61 | 219.0 (49.1) | 248.2 (101.2) | 27.0 | (-5.8, 59.8) | | | |
| DHT (ng/dL) | 12 | TRT | 420 | 15.6 (7.5) | 69.1 (62.9) | 53.3 | (49.1, 57.5) | 51.4 | (45.5, 57.4) | <.001 |
| | | Placebo | 413 | 15.8 (6.8) | 17.7 (9.2) | 1.9 | (-2.4, 6.1) | | | |
| | 36 | TRT | 231 | 16.0 (8.0) | 61.3 (59.7) | 45.0 | (39.6, 50.3) | 41.0 | (33.4, 48.5) | |
| | | Placebo | 242 | 15.3 (6.2) | 19.2 (9.9) | 4.0 | (-1.3, 9.3) | | | |
| Estradiol (pg/mL) | 12 | TRT | 418 | 20.8 (8.1) | 32.6 (23.2) | 11.7 | (10.1, 13.4) | 11.8 | (9.4, 14.1) | <.001 |
| | | Placebo | 402 | 20.8 (7.5) | 20.8 (9.9) | -0.0 | (-1.7, 1.6) | | | |
| | 36 | TRT | 235 | 20.3 (7.5) | 29.2 (17.2) | 9.0 | (7.4, 10.6) | 7.6 | (5.3, 9.9) | |
| | | Placebo | 237 | 21.1 (7.3) | 22.3 (9.9) | 1.4 | (-0.2, 3.0) | | | |

3

4 **Legend.** Means and standard deviations (SD) are presented for baseline and each visit
5 for subjects with a measurement at the visit. Least Square Means (LS Means) estimates
6 of change from baseline (95% confidence interval (CI)) for each group and the
7 difference between TRT and placebo at each visit are derived from a linear mixed
8 effects model controlling for baseline value and pre-existing CVD, assuming an
9 unstructured covariance matrix. The omnibus test p value (P) is a test of the null
10 hypothesis of no difference between TRT and placebo groups across all time points. To
11 convert serum total testosterone concentrations in nanograms per deciliter to
12 nanomoles per liter, multiply testosterone concentration in nanograms per deciliter by
13 0.0347. To convert estradiol concentrations from picogram per milliliter to picomoles per
14 liter, multiply estradiol concentrations in picogram per milliliter by 3.67. To convert

- 1 dihydrotestosterone concentrations in nanograms per deciliter to nanomoles per liter,
- 2 multiply dihydrotestosterone concentrations in nanograms per deciliter by 0.0344.
- 3 Yr, years; CVD, cardiovascular disease; PDQ-4, question 4 of the Psychosexual Diary
- 4 Questionnaire; HIS-Q, Hypogonadism Impact of Symptoms Questionnaire; PDE5
- 5 Inhibitor, Selective phosphodiesterase 5 inhibitor
- 6

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Figure Legends

Figure 1. Model-estimated changes from baseline in overall sexual activity (PDQ-4 score) in participants enrolled in the Sexual Function Study.

Legend: A linear mixed model was fit with fixed effects for treatment, visit, and treatment-visit interaction, baseline PDQ-4 score and pre-existing cardiovascular disease and a random per-subject repeated measures effect with an unstructured covariance matrix. The mean and the corresponding 95% confidence intervals are shown by treatment group and time point. The omnibus test p value from a test of the null hypothesis of no difference between the TRT and placebo groups across all time points is shown.

Figure 2. Model-estimated changes from baseline in hypogonadal symptoms (Composite HIS-Q score) (Panel A, top), sexual symptoms (Sexual symptom domain of HIS-Q) (Panel B, middle) and sexual desire (HIS-Q libido subdomain score) (Panel C, bottom) in men enrolled in the Sexual Function Study.

Legend: A linear mixed model was fit to each of the three scores, separately, with fixed effects for treatment, visit, and treatment-visit interaction, corresponding baseline score and pre-existing cardiovascular disease and a random per-subject repeated measures effect with an unstructured covariance matrix. The mean and the corresponding 95% confidence intervals are shown by treatment group and time point. The omnibus test p values were derived separately for each score and were derived from a test of the null hypothesis of no difference between the TRT and placebo groups across all time points.

Figure 3. Model-based estimates of Patient Global Impression of Change - Libido score at each visit

Legend: A linear mixed model was fit with fixed effects for treatment, visit, and treatment-visit interaction and pre-existing cardiovascular disease and a random per-subject repeated measures effect with an unstructured covariance matrix. Lower scores on HIS-Q indicate improvement (fewer symptoms). The mean and the corresponding 95% confidence intervals are shown by treatment group and time point. The omnibus test p value from a test of the null hypothesis of no difference between the TRT and placebo groups across all time points is shown.

Figure 4. Model-estimated change from baseline in erectile function, assessed using IIEF-5, in men enrolled in the Sexual Function Study.

Legend: A linear mixed model was fit with fixed effects for treatment, visit, and treatment-visit interaction, baseline value, pre-existing cardiovascular disease and a random per-subject repeated measures effect with an unstructured covariance matrix. The mean and the corresponding 95% confidence intervals are shown by treatment group and time point. The omnibus test p value from a test of the null hypothesis of no difference between the TRT and placebo groups across all time points is shown.

Figure 5. Model-based estimates of change in HIS-Q and its Sexual function and Libido subdomain scores in all TRAVERSE participants (Panels A, B, and C respectively).

Legend: A linear mixed model was fit to each of the three scores, separately, with fixed effects for treatment, visit, and treatment-visit interaction, corresponding baseline value and pre-existing cardiovascular disease and a random per-subject repeated measures effect with an unstructured covariance matrix. Lower scores on HIS-Q indicate improvement (fewer symptoms). The mean and the corresponding 95% confidence intervals are shown by treatment group and time point. The omnibus test p values were derived separately for each score and were derived from a test of the null hypothesis of no difference between the TRT and placebo groups across all time points.

Figure 6. Preplanned subgroup analyses of the PDQ-4 by CVD risk (Panels A and B), age group (65 years or older, < 65 years, Panels C and D), baseline testosterone level (< 250 ng/dl; 250 ng/dl or higher; Panel E and F) and race (Black, White; Panels G and H).

Legend.

Four separate linear mixed models were fit with fixed effects for treatment, visit, subpopulation variable (prior CVD risk group, age, testosterone level or race), corresponding two- and three-way interaction terms, baseline PDQ-4 value and a random per-subject repeated measures effect with an unstructured covariance matrix. The models with age, testosterone level or race also included pre-existing cardiovascular disease as a covariate. The LS Means and the corresponding 95%

confidence intervals are shown by treatment group and time point for each age group. The omnibus test p value from a test of the null hypothesis of no difference between the TRT and placebo groups across all time points for each age group is shown. The p value for the contrast test of interaction that tested if there is a difference in treatment effect between the two CVD risk groups was 0.421; age groups was 0.338, baseline testosterone levels was 0.089 and race was 0.822.

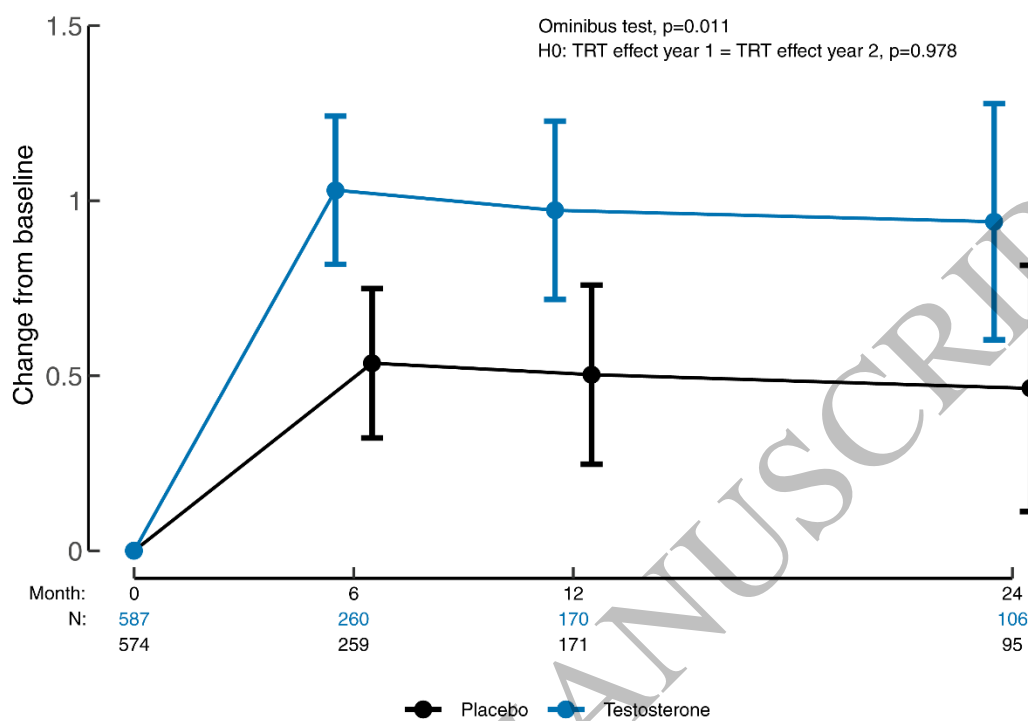


Figure 1
152x108 mm (x DPI)

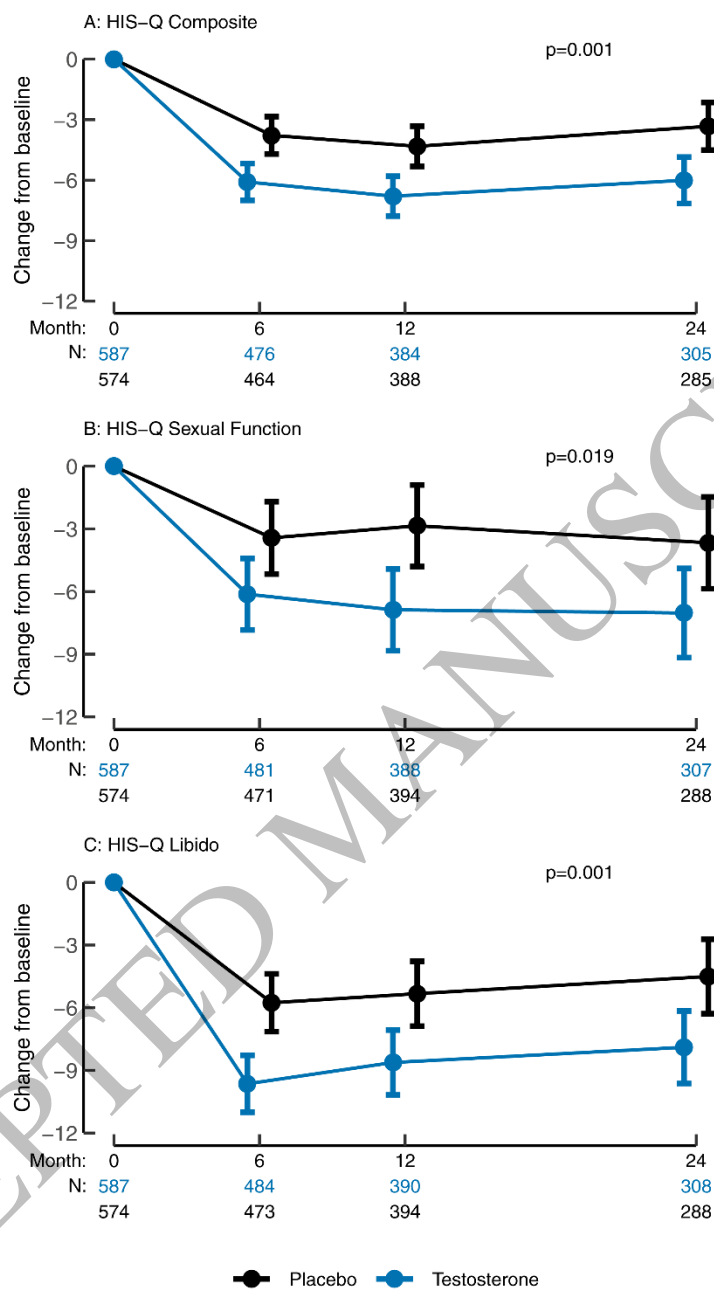


Figure 2
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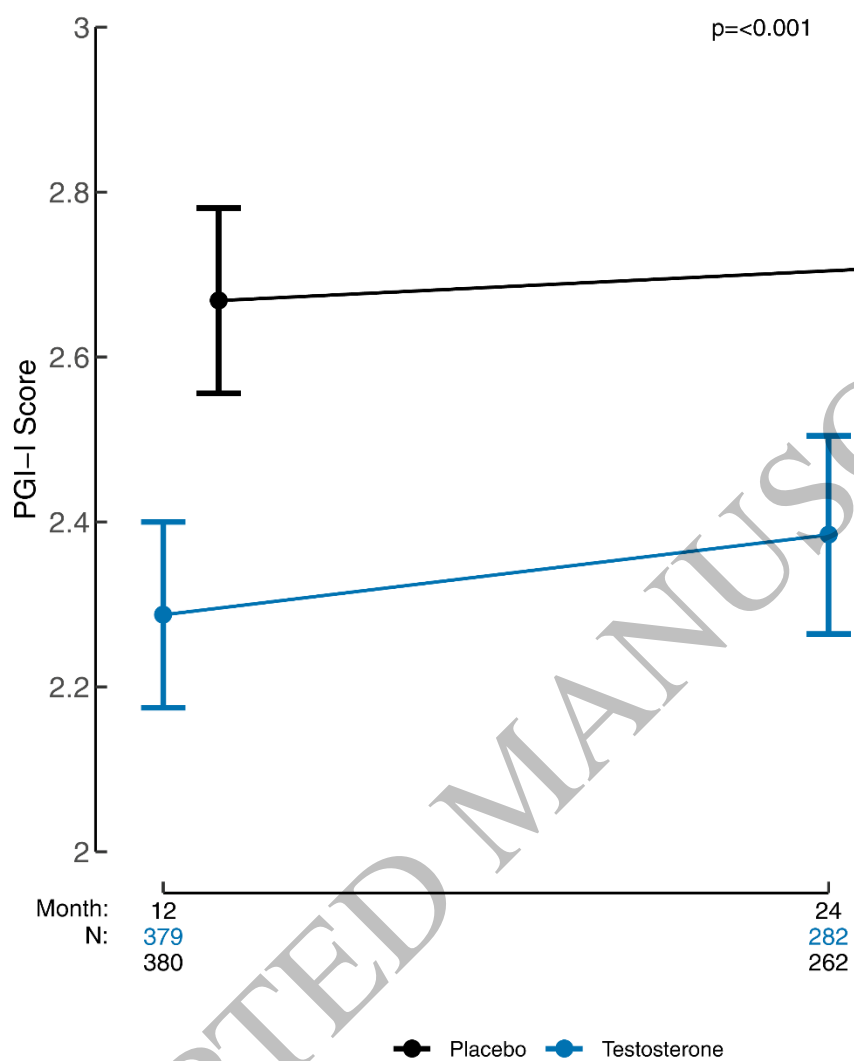


Figure 3
152x152 mm (x DPI)

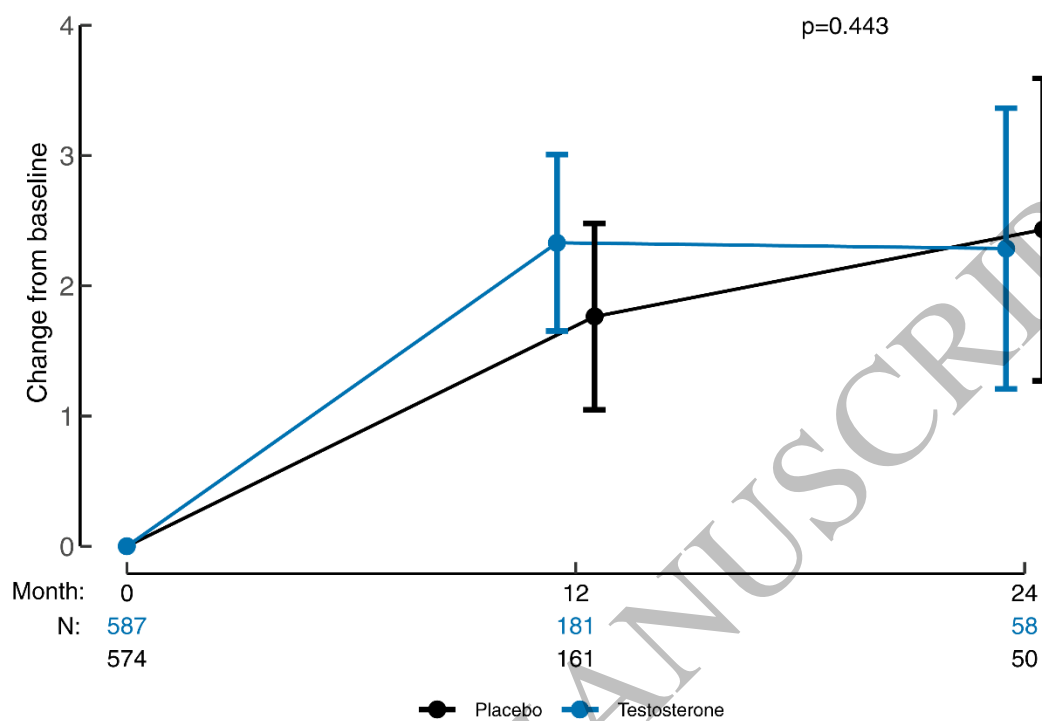


Figure 4
152x108 mm (x DPI)

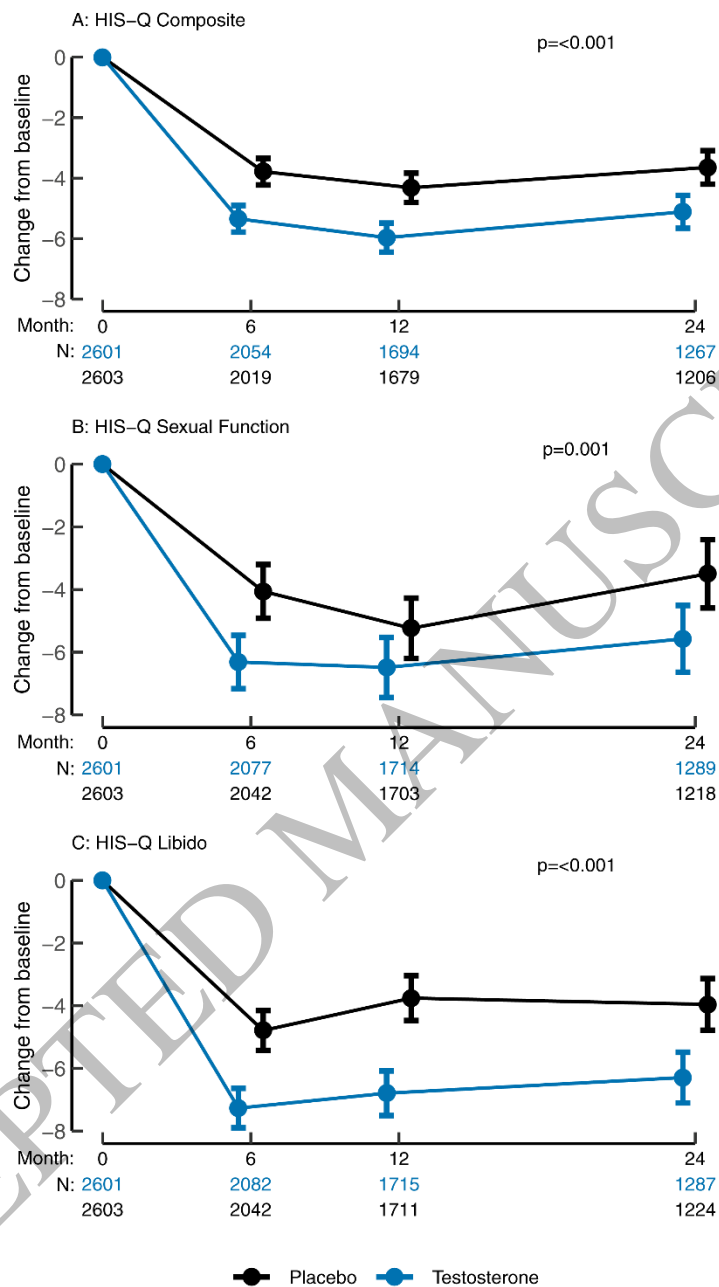


Figure 5
107x178 mm (x DPI)

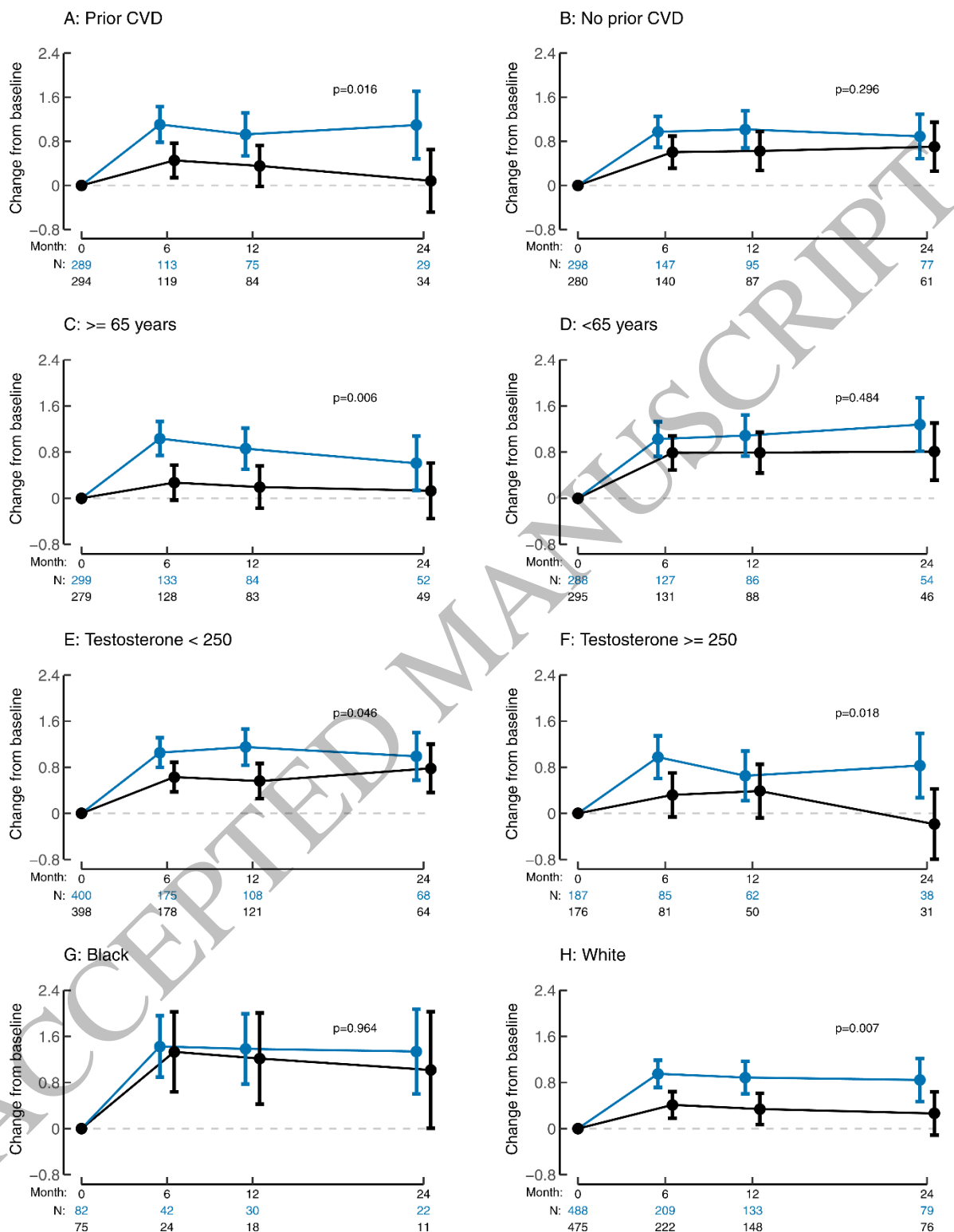


Figure 6
165x206 mm (x DPI)