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# Association of Sex Hormones With Sexual Function, Vitality, and Physical Function of Symptomatic Older Men With Low Testosterone Levels at Baseline in the Testosterone Trials

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## Abstract

### Context:

The prevalence of sexual dysfunction, low vitality, and poor physical function increases with aging, as does the prevalence of low total and free testosterone (TT and FT) levels. However, the relationship between sex hormones and age-related alterations in older men is not clear.

### Objective:

To test the hypotheses that baseline serum TT, FT, estradiol (E2), and sex hormone-binding globulin (SHBG) levels are independently associated with sexual function, vitality, and physical function in older symptomatic men with low testosterone levels participating in the Testosterone Trials (TTrials).

### Design:

Cross-sectional study of baseline measures in the TTrials.

### Setting:

The study was conducted at 12 sites in the United States.

### Participants:

The 788 TTrials participants were ≥ 65 years and had evidence of sexual dysfunction, diminished vitality, and/or mobility disability, and an average of two TT < 275 ng/dL.

### Interventions:

None.

### Main Outcome Measures:

Question 4 of Psychosocial Daily Questionnaire (PDQ-Q4), the FACIT-Fatigue Scale, and the 6-minute walk test.

### Results:

Baseline serum TT and FT, but not E2 or SHBG levels had small, but statistically significant associations with validated measures of sexual desire, erectile function, and sexual activity. None of these hormones was significantly associated within or across trials with FACIT-Fatigue, PHQ-9 Depression or Physical Function-10 scores, or gait speed.

### Conclusions:

FT and TT levels were consistently, independently, and positively associated, albeit to a small degree, with measures of sexual desire, erectile function, and sexual activity, but not with measures of vitality or physical function in symptomatic older men with low T who qualified for the TTrials.

Sexual function (1, 2), vitality, and physical function (3) decline with aging, as do total and free testosterone levels (4,–6). The relationships between sex hormone levels and sexual function, vitality, and physical function in symptomatic older hypogonadal men are unclear.

Population-based studies indicate that sexual function and activity are affected by age and to a lesser degree testosterone levels. Age, but not total testosterone (TT), was associated with sexual activity in the Baltimore Longitudinal Study of Aging (7). The Olmstead Longitudinal Study of 414 men found an association between TT and erectile function but not sexual desire after adjusting for age (8). Bioavailable T was not associated with erectile function or sexual desire after adjustment for age in the REDUCE study population (9). Low serum TT levels were found to be poor predictors of sexual function, whereas age, BMI, and International Prostate Symptom Score were significant predictors of sexual desire, erectile function, and sexual activity (9). TT and free testosterone (FT), but not estradiol (E2) or dihydrotestosterone (DHT) were associated with sexual function in middle-aged and older men in the European Male Aging Study (10).

Participants in the TTrials are well-characterized, community-dwelling, older men with well-defined symptoms, a restricted range of low T levels (excluding both severely low and borderline low T levels), and relatively healthy (exclusion of significant age-related comorbid illnesses). Baseline information obtained in evaluating the participants who qualified for these trials provides an opportunity to test the hypotheses that baseline serum TT and FT, E2 and sex hormone-binding globulin (SHBG) levels are independently associated with sexual function, vitality, and physical function in symptomatic men ≥ 65 years of with low testosterone levels. This study enrolled a large number of symptomatic men with low TT and FT levels and used validated instruments to assess sexual function, vitality, and physical function.

## Materials and Methods

### Participants

To qualify for the TTrials, men had to be ≥ 65 years old and have self-reported sexual dysfunction, diminished vitality, and/or mobility limitation (11). Men were required to have two early morning TT values with an initial TT < 275 ng/dL, a second < 300 ng/dL, and an average < 275 ng/dL during screening visits prior to randomization. Men who had a serum testosterone concentration < 100 ng/dL were excluded, unless evaluation did not show either hypopituitarism or a known cause of primary hypogonadism. They qualified for the Sexual Function Trial if they complained of reduced libido, scored ≤ 20 on the Sexual Desire Domain of the Derogatis Interview for Sexual Function (DISF-SDD) (12), and had a partner willing to have sexual intercourse at least twice a month. Men were enrolled in the Vitality Trial if they reported decreased energy and scored < 40 on the FACIT-Fatigue scale (13). Men were enrolled in the Physical Function Trial if they had self-reported difficulty walking one-quarter mile and/or walking up one flight of stairs and a gait speed of < 1.2 m/s on the 6-minute walk test (14, 15). Men who qualified, could participate in more than one trial. These primary outcome measures were collected in all men who qualified for the study. However, the primary analysis of the association of hormone levels and outcome measures was restricted to the group of men who qualified for the specific trials.

### Assays

TT (6) and E2 levels (16) were measured by liquid chromatography and tandem mass spectrometry (LC-MS/MS), and FT was assayed by equilibrium dialysis in the Brigham Research Assay Core Laboratory (BRAC), Boston, MA (17). Testosterone was extracted by solid phase extraction, separated by high performance liquid chromatography and mass spectrometry, and determined by mass spectrometry in electrospray ionization ($ESI^+$) source. Deuterated testosterone was as an internal standard for the calibration of assay. M/E ratios of 109 and 97 were used for quantitation. The lower limit of quantitation is 1 ng/dL and interassay CV < 7%.

E2 was extracted by solid phase extraction, derivatized, separated by high performance liquid chromatography, and measured by tandem mass spectrometry in electrospray ionization ($ESI^+$) source and multiple reaction monitoring (MRM) of transitions (M/E 506.4/170.9). Deuterated E2 was used as an internal standard for the calibration of assay. The lower limit of quantitation was 1 pg/mL; the interassay coefficients of variation were 10.1%, 8.5%, and 6.3%, respectively, in QC pools with E2 concentrations of 8 pg/mL, 57.1 pg/mL, and 157.0 pg/mL, respectively.

SHBG was assayed by a two-site immunochemiluminescence assay (Beckman-Coulter, Inc.) whose lower limit of quantitation was 0.3 nmol/L.

### Functional Assessments

Validated questionnaires were used in each of the three trials. Sexual desire was assessed at baseline with the DISF-SDD. Erectile function was evaluated by the Erectile Function Domain of the International Index of Erectile Function (IIEF-EFD) (18), sexual activity over 7 days was measured using question 4 of the Psychosexual Daily Questionnaire (PDQ) (19). A score of < 20 on the DISF-SDD is considered a significant reduction in sexual desire, and a score of < 22 on the IIEF-EFD is considered a significant reduction in erectile function (19). Vitality was assessed with the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-Fatigue) (20), the Positive and Negative Affect Schedule (PANAS) (21), and the Patient Health Questionnaire (PHQ-9) (22) questionnaires. Physical function was evaluated by determining gait speed during a 6 minute walk (15) and with the Physical Function Subscale (PF-10) of the Medical Outcomes Study Short Form-36 (MOS SF-36) Health Survey Questionnaire(23). The ranges of scores for the tests of efficacy were limited by the ranges required to qualify for each trial.

### Statistical Analysis

We ran the analyses using SAS/STAT, version 9.4. We used multivariable linear regression, adjusting for confounding factors that were prespecified separately for each trial, to evaluate the associations between baseline serum TT, FT, E2, and SHBG levels and sexual desire (DISF-SDD), erectile function (IIEF-EFD) and sexual activity (Question 4 of the PDQ). The prespecified confounding factors for the Sexual Function Trial were BMI, age, hypertension, diabetes, and waist circumference and for the Physical Function and Vitality Trials were age and BMI. Similar analyses were done to evaluate the association between these hormonal measures and the total scores on the FACIT-Fatigue, PANAS, PHQ-9, PF-10 questionnaires, and gait speed during a 6 minute walk. All of the analyses were prespecified.

We did not correct for multiple comparisons due to the correlation among function measures, particularly within each trial, which would have made such corrections overly conservative (24). Consequently, our false positive rate is somewhat inflated.

## Results

We evaluated 470 older men with low TT levels in the Sexual Function Trial, 474 men in the Vitality Trial, and 390 men in the Physical Function Trial of the TTrials (Table 1). Men who qualified for the Physical Function Trial were somewhat older (73.3 ± 6.2 years) than those in the other two trials (71.6 ± 5.3 and 71.9 ± 5.8 years). However, mean BMIs and mean TT, FT, E2 and SHBG levels were similar in the three trials. Over 70% of participants in all three trials had a history of hypertension. More participants in the Physical Function Trial had a history of diabetes (42.6%) as compared with 35.0% in the Sexual Function Trial and 35.7% in the Vitality Trial. As expected, the mean scores on the DISF-SDD were lower (11.8 ± 6.6) for the men in the Sexual Function Trial when compared with those who qualified for the Physical Function (14.3 ± 8.1) and Vitality (14.0 ± 7.9) Trials. Men who qualified for the Vitality Trial had a mean score on the FACIT Fatigue scale of 31.4 ± 6.4 vs. 37.8 ± 8.9 and 37.5 ± 8.4 for the Sexual Function and Physical Function Trials, respectively. Mean gait speed was slower in the Physical Function Trial (0.96 ± 0.19 m/s) when compared with the Sexual Function (1.1 ± 0.2 m/s) and Vitality (1.1 ± 0.2 m/s) Trials.

Table 1.

Participants' Characteristics and Baseline Measurements

| Trial | Sexual Function | Vitality | Physical Function | Overall[b] |
|---|---|---|---|---|
| N | 470 | 474 | 390 | 788 |
| Age (y)[a] | 71.6 ± 5.3 | 71.9 ± 5.8 | 73.3 ± 6.2 | 72.2 ± 5.7 |
| Hypertension (%) | 71.3 | 73.0 | 73.9 | 71.5 |
| Diabetes (%) | 35.0 | 35.7 | 42.6 | 36.9 |
| BMI | 31.0 ± 3.5 | 31.0 ± 3.6 | 31.6 ± 3.4 | 31.0 ± 3.5 |
| WC | 110 ± 10.7 | 110.7 ± 10.4 | 112.4 ± 11.3 | 110.5 ± 10.8 |
| TT (ng/dL) | 238.2 ± 69.0 | 237.3 ± 70.4 | 235.1 ± 67.4 | 238.1 ± 68.9 |
| FT (pg/mL) | 58.3 ± 20.2 | 58.1 ± 20.2 | 57.0 ± 20.2 | 58.2 ± 20.3 |
| E2 (pg/mL) | 22.6 ± 9.7 | 23.2 ± 9.9 | 23.4 ± 9.0 | 23.1 ± 9.7 |
| SHBG (nmol/L) | 30.6 ± 15.5 | 31.6 ± 17.1 | 32.0 ± 16.6 | 31.2 ± 16.1 |
| DISF-SDD | 11.8 ± 6.6 | 14.0 ± 7.9 | 14.3 ± 8.1 | 14.0 ± 7.8 |
| IIEF-EFD | 8.0 ± 8.2 | 7.4 ± 7.8 | 6.9 ± 7.7 | 7.9 ± 8.2 |
| PDQ | 1.4 ± 1.3 | 1.5 ± 1.4 | 1.4 ± 1.3 | 1.5 ± 1.4 |
| FACIT-Fatigue | 37.8 ± 8.9 | 31.4 ± 6.4 | 37.5 ± 8.4 | 36.9 ± 8.7 |
| PHQ-9 | 4.9 ± 3.9 | 6.6 ± 4.0 | 5.5 ± 4.0 | 5.4 ± 4.0 |
| Gait speed (m/s) | 1.1 ± 0.2 | 1.1 ± 0.2 | 0.96 ± 0.19 | 1.1 ± 0.2 |
| PF-10 | 75.6 ± 20.4 | 70.4 ± 19.9 | 65.4 ± 20.4 | 72.1 ± 20.6 |

Abbreviations: BMI, body mass index; WC, waist circumference; Total T, total testosterone; Free T, free testosterone; E2, estradiol; DISF-SDD, Derogatis Interview for Sexual Function-Sexual Desire Domain; PF-10, Physical Function Subscale.

[a]Mean ± SD.
[b]Seven hundred ninety men were enrolled, but 2 were deemed ineligible and are not included here.

Scatter plots illustrate the unadjusted and adjusted associations between TT levels and sexual desire, erectile function, and sexual activity for men who qualified for the Sexual Function Trial (Figure 1). Similar plots are shown for the Vitality (Figure 2) and Physical Function (Figure 3) Trials. Mean sexual desire (DISF; P = .03), erectile function (IIEF; P = .004), and sexual activity (PDQ-Q4; P = .02) increased significantly with TT (Table 2). An increase of 70 ng/dL [one standard deviation (SD)] in TT was associated with a 0.63-point increase in average DISF score, a 1.12-point increase on the IIEF, and a 0.14-point increase on the PDQ-Q4. Sexual function measures were more strongly associated with FT. Mean sexual desire (P = .02), erectile function (P = .005), and sexual activity (P = .01) increased with FT (Table 2). A 20 pg/mL (1 SD) increase in FT was associated with an increase of 0.74 on the DISF, 1.12 on the IIEF, and 0.16 on the PDQ-Q4. Estradiol and SHBG were not significantly associated with these measures of sexual function.

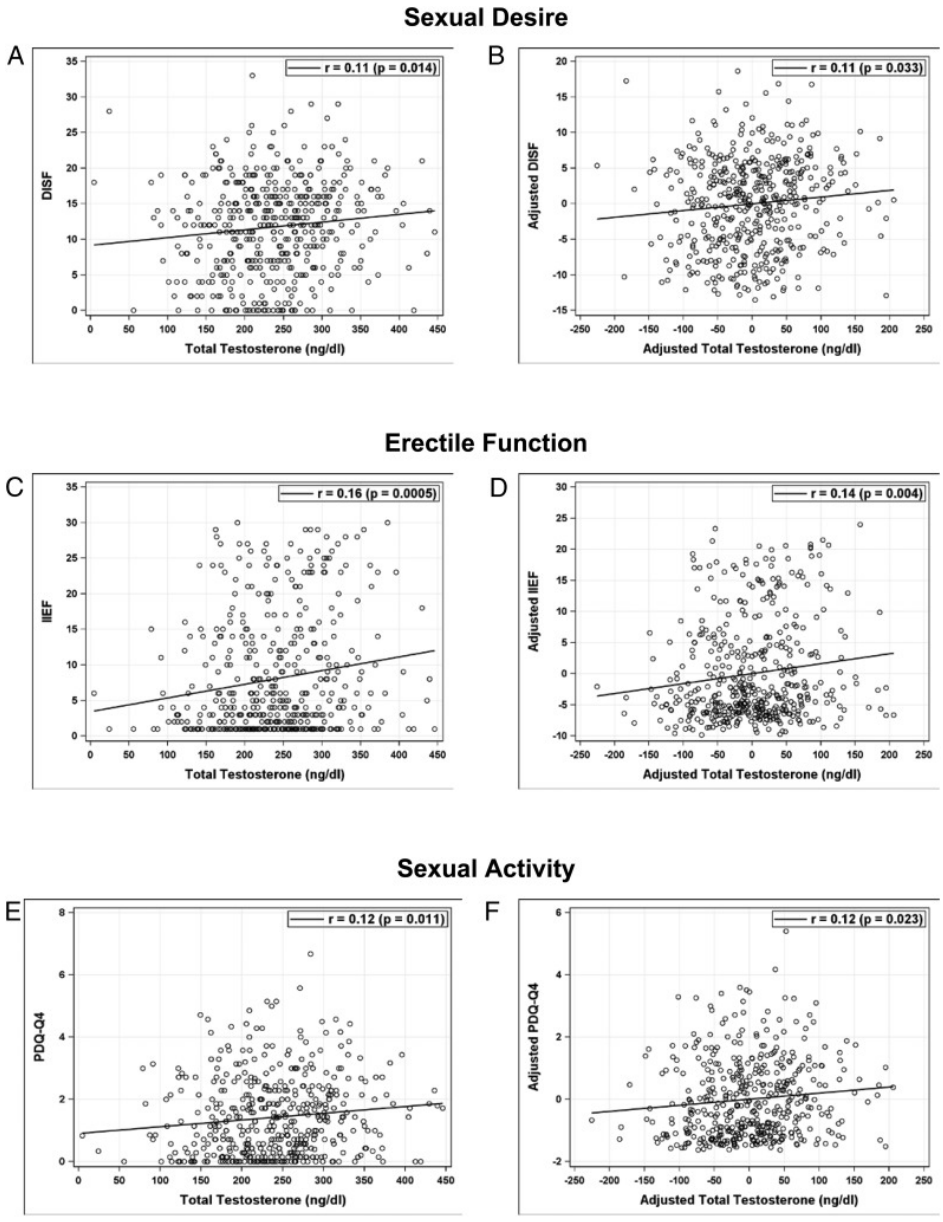


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Figure 1.

Association of total testosterone (TT) with sexual function parameters at the baseline visit in men enrolled in the Sexual Function Trial of the TTrials. A, Positive association of TT (unadjusted) with sexual desire [Derogatis Inventory of Sexual Function (DISF)] (unadjusted); B, adjusted TT with adjusted DISF; C, erectile function [International Index of Erectile Function (IIEF) (unadjusted) with total testosterone (unadjusted); D, adjusted T with IIEF; E, TT (unadjusted) with sexual activity [Psychosexual Daily Questionnaire, Question 4 (PDQ-Q4) (unadjusted); and F, adjusted TT with adjusted PDQ-Q4. The adjustments were made for the potential confounders of age, hypertension, diabetes, waist circumference and BMI. The adjusted values shown are those that remained after removing the effects due to the potential confounders.

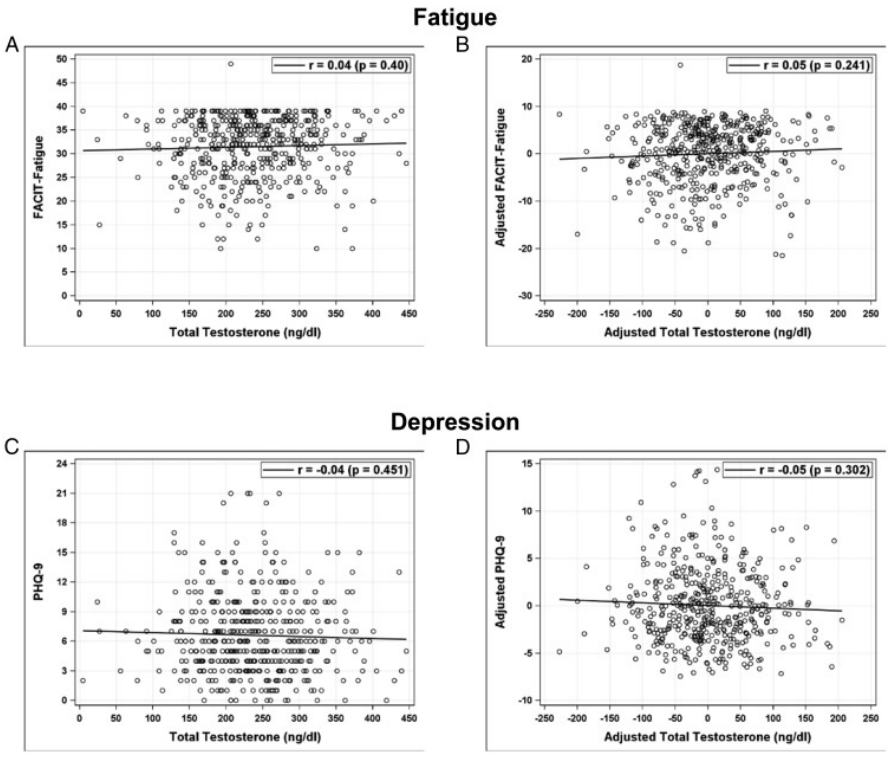


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Figure 2.

Association of vitality and depression with total testosterone at the baseline visit in men enrolled in the Vitality Trial of the TTrials. A, Association of total testosterone (TT) (unadjusted) with vitality assessed by the FACIT-Fatigue scale (unadjusted); B, adjusted FACIT-Fatigue with adjusted TT; C, TT (unadjusted) with depression as assessed by the PHQ-9 (unadjusted); and D, adjusted TT with adjusted PHQ-9. The adjustments were for the potential confounders of age and BMI. The adjusted values shown are those that remained after removing the effects due to the potential confounders.

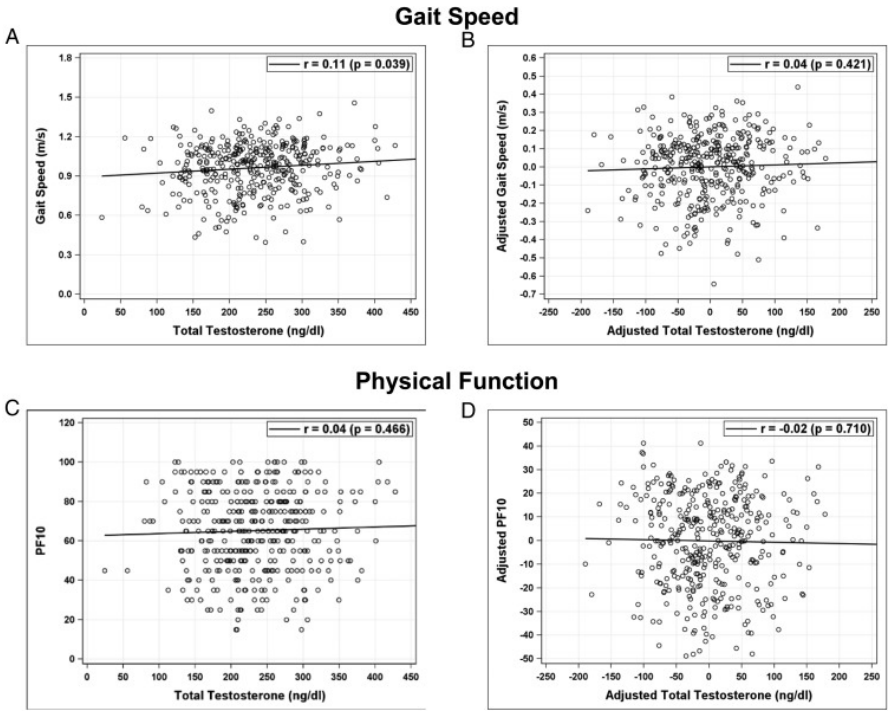


Figure 3.

Association of total testosterone (TT) with physical function parameters at the baseline visit in men enrolled in the Physical Function Trial of the TTrials. A, Association of TT (unadjusted) with gait speed (unadjusted); B, adjusted TT with adjusted gait speed; C, TT (unadjusted) with the physical function domain of the SF-36 (PF10) (unadjusted); and D, adjusted TT with the adjusted PF10. The adjustments were for age and BMI. The adjusted values shown are those that remained after removing the effects due to the potential confounders.

Table 2.

Association of Hormones With Sexual Function, Vitality, and Physical Function

| Outcome | Total T | | Free T | | Estradiol | | SHBG | |
|---|---|---|---|---|---|---|---|---|
| | ß (95% CI) | P | ß (95% CI) | P | ß (95% CI) | P | ß (95% CI) | P |
| Sexual Function[a] | | | | | | | | |
| (Sexual Function Trial Only) | | | | | | | | |
| DISF | 0.009 (0.001, 0.018) | 0.034 | 0.037 (0.006, 0.068) | 0.020 | 0.035 (−0.028, 0.097) | 0.275 | −0.016 (−0.059, 0.023) | 0.469 |
| IIEF | 0.016 (0.005, 0.027) | 0.004 | 0.056 (0.017, 0.094) | 0.005 | 0.047 (−0.030, 0.124) | 0.230 | −0.049 (−0.102, 0.005) | 0.074 |
| PDQ-Q4 | 0.002 (0.000, 0.004) | 0.024 | 0.008 (0.002, 0.014) | 0.014 | 0.012 (−0.000, 0.024) | 0.051 | −0.006 (−0.014, 0.003) | 0.185 |
| Sexual Function[a] | | | | | | | | |
| (All T-Trial Participants) | | | | | | | | |
| DISF | 0.008 (−0.000, 0.016) | 0.055 | 0.048 (0.020, 0.077) | <0.001 | 0.060 (0.003, 0.117) | 0.039 | −0.047 (−0.085, −0.009) | 0.016 |
| IIEF | 0.014 (0.004, 0.246) | 0.005 | 0.049 (0.013, 0.085) | 0.008 | 0.047 (−0.023, 0.122) | 0.218 | −0.046 (−0.097, 0.004) | 0.073 |
| PDQ-Q4 | 0.002 (0.000, 0.003) | 0.026 | 0.008 (0.003, 0.013) | 0.003 | 0.012 (0.002, 0.022) | 0.022 | −0.006 (−0.013, 0.001) | 0.078 |
| Vitality Trial[b] | | | | | | | | |
| FACIT Fatigue | 0.005 (−0.003, 0.013) | 0.217 | 0.000 (−0.030, 0.031) | 0.982 | 0.001 (−0.058, 0.061) | 0.962 | 0.016 (−0.022, 0.054) | 0.401 |
| PHQ-9 | −0.003 (−0.008, 0.002) | 0.290 | −0.006 (−0.026, 0.013) | 0.527 | −0.009 (−0.046, 0.029) | 0.657 | 0.012 (−0.013, 0.036) | 0.347 |
| Physical Function Trial[b] | | | | | | | | |
| Gait Speed (m/s) | 0.000 (−0.000, 0.000) | 0.314 | 0.001 (−0.000, 0.002) | 0.231 | −0.002 (−0.004, 0.000) | 0.104 | −0.001 (−0.002, 0.000) | 0.112 |
| PF-10 | −0.003 (−0.033, 0.027) | 0.824 | 0.004 (−0.100, 0.108) | 0.938 | −0.311 (−0.530, −0.092) | 0.006 | −0.068 (−0.199, 0.063) | 0.308 |

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Abbreviations: DISF, Derogatis Interview for Sexual Function-Sexual Desire Domain.

[a]Adjusted for BMI, age, hypertension, diabetes, and waist circumference.
[b]Adjusted for age and BMI.

The associations of TT, FT, E2, and SHBG with these measures of sexual function for all participants in the TTrials were similar to those for men who were enrolled in the Sexual Function Trial (Table 2). TT was marginally associated with desire (DISF; P = .055), and significantly associated with erectile function (IIEF; P = .005) and sexual activity (PDQ; P = .03). The associations, as reflected by the magnitude of the regression coefficients, were stronger for FT: sexual desire (DISF; P < .001), erectile function (IIEF; P = .008), and sexual activity (PDQ; P = .003). In contrast to our findings for the participants in the Sexual Function Trial, serum E2 levels were associated with sexual desire (P = .04) and sexual activity (P = .02) for all participants. However, the serum SHBG levels were inversely associated with sexual desire (P = .02).

After adjustment for age and BMI, the associations between these hormone measurements and the objective measures of vitality and physical function were not statistically significant for men who qualified for the Vitality and Physical Function Trials, respectively. As shown in Table 2, none of the hormones measured (TT FT, E2 or SHBG) was significantly associated with either FACIT-Fatigue or PHQ-9 scores. Similarly, none of the hormones measured (TT, FT, SHBG) was associated with gait speed or PF-10 scores, but E2 was inversely associated with PF-10 scores (Table 2). Results were similar in the overall cohort of 788 men.

## Discussion

We observed consistent and statistically significant, although small, positive cross-sectional associations between baseline serum TT and FT levels and measures of sexual desire, erectile function, and sexual activity in our clinical trial population of 470 symptomatic men, ≥ 65 years who had low T levels and who qualified for the Sexual Function Trial and for the entire group of 788 men who qualified for at least one of the three trials. Serum E2 levels were associated with sexual desire and sexual activity for all participants, but were not associated with any of the measures of sexual function in participants who qualified for the Sexual Function Trial. The magnitude of the association of serum estradiol with sexual activity among all participants, however, was equivalent to that among Sexual Trial participants (adjusted ß = 0.012 Sexual Function Trial and among all participants), which suggests that statistical significance was due to the larger sample. SHBG levels were not associated with any of these measures for participants in the Sexual Function Trial, and SHBG was inversely associated with sexual desire for the complete cohort of men who qualified for at least one trial. These baseline hormonal measures were not associated with baseline FACIT Fatigue or PHQ-9 scores in the Vitality Trial or with gait speed or PF-10 scores in the Physical Function Trial.

While statistically significant at the 0.05 level, the magnitude of the associations between baseline serum TT and FT and sexual desire, erectile function, and sexual activity was small, which is to be expected given the trial eligibility-driven restricted range of the values being correlated. Indeed, this issue, and the cross sectional nature of the comparisons reported here, likely contributed to the nonsignificant correlations between hormonal measures and trial outcome measures of vitality, depression, physical function, and gait speed. Our results should be interpreted cautiously, given the number of statistical comparisons, when considering our lack of adjustment for multiple comparisons. The number of significant findings in the Sexual Function Trial, however, exceeds that which would be expected by chance alone. Ultimately, the results of testosterone treatment in the TTrials will reveal the extent to which TT and FT are correlated with vitality, depressive symptoms, sexual function, physical function, and gait speed.

Low TT levels are associated with reduced sexual activity in younger men, and sexual function is improved when TT levels are restored (25,–27); but the relationship of testosterone levels with sexual activity in older men has been controversial (7,–10, 28). Many studies report reduced sexual activity in older men with both low and normal TT levels. Comorbidities and medications are thought to account for reduced sexual activity in many older men. Using a long-acting GnRH agonist to suppress endogenous LH and TT levels coupled with graded doses of testosterone supplementation in men 60–75, Gray and co-workers observed that FT levels during treatment were positively associated with overall sexual function (P = .001), waking erections (P = .040), spontaneous erections (P = .047), and libido (P = .027), but not with intercourse frequency (P = .43) or masturbation frequency (P = .81) (29). While placebo-controlled studies have reported testosterone-related improvement in sexual function in older men with low TT levels (30, 31), the number of men in these trials has been relatively small and none has used LC-MS/MS for TT, FT, and E2 and validated measurements of sexual desire, erectile function, and sexual activity.

We used a commercial laboratory for measuring TT at the screening visits because of the need for rapid turnaround of results, but we used a research laboratory for the baseline hormonal and SHBG levels reported here. Both laboratories participate in the CDC program to harmonize testosterone assays. Furthermore, the LC-MS/MS assay for TT had a lower limit of quantitation of 1 ng/dL and a high level of precision in the low range. Nonetheless, approximately 10% of participants who qualified for the T Trial had a TT value > 300 ng/dL at the baseline visit, likely reflecting a “regression to the mean” effect, as has been reported previously (32).

Previous reports suggest that sexual desire and erectile function are decreased when the serum TT is near the lower limit of the normal range (33,–36). Although most of our participants had baseline TT values that were in the 200–300 ng/dL range, we were not able to identify a definite threshold for either TT or FT, likely because the range of testosterone levels was limited. Others also have failed to identify symptom-specific T thresholds when age was considered (37).

The possibility that E2 contributes to sexual function in men is uncertain. Using a GnRH agonist to suppress endogenous testosterone levels in normal men ages 20–50 years and providing varying amounts of testosterone gel with or without an aromatase inhibitor, one study reported that both E2 and testosterone contribute to maintenance of normal male sexual function (27). However, a small number of men with very low or undetectable levels of E2 due to aromatase deficiency reportedly have sexual desire and penile erections (38, 39). Libido and sexual activity are reported to increase when these men are treated with estrogen and testosterone levels are maintained in the normal adult male range. Similarly, a man with estrogen resistance due to a mutation of the estrogen receptor alpha reported strong heterosexual interests, morning erections and nocturnal emissions (40). We did not find associations between E2 levels and sexual desire, erectile function or sexual activity in the men who qualified for the Sexual Function Trial. However, E2 levels were weakly associated with sexual desire (P = .09) in the larger group of all T Trial participants. E2 levels, of course, are lower in men who have low testosterone levels, so this may have limited our ability to detect associations of E2 and measures of sexual function. It may be that higher estrogen levels in the presence of testosterone contribute to sexual function.

Our inability to find an association between TT or FT and objective measures of low vitality or poor physical function in men who qualified for the Vitality or Physical Function Trial could be due to our enrollment criteria. This trial was limited to men 65 and older who had low serum TT levels, symptoms, and objective evidence of reduced vitality, sexual and/or physical function. Thus, the ranges of baseline testosterone levels and scores on the objective responses were constrained.

The participants were 788 well-characterized, community-dwelling, older men with well-defined symptoms, a restricted range of low TT levels (excluding both severely low and borderline low as well as normal TT levels). They were relatively healthy (exclusion of significant age-related comorbid illnesses). The limitations of the study include the restricted range of TT levels and restricted ranges of sexual function, vitality and physical function, which were responsible for relatively small effect sizes.

We conclude that baseline FT and TT, but not E2 or SHBG, are consistently and independently associated with measures of sexual desire, erectile function and sexual activity in older men with low testosterone levels and symptoms of sexual dysfunction. These findings suggest that the circulating FT and TT levels contribute more than E2 or SHBG to the variation in some measures of sexual dysfunction in older men with low TT. In contrast, we did not find an association between baseline FT, TT, E2 or SHBG and measures of vitality or physical function in the participants who qualified for those trials.

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## Footnotes

Abbreviations:

BRAC Brigham Research Assay Core Laboratory
DHT dihydrotestosterone
$ESI^+$ electrospray ionization
E2 estradiol
FACIT-Fatigue Functional Assessment of Chronic Illness Therapy-Fatigue
FT free testosterone
IIEF-EFD Erectile Function Domain of the International Index of Erectile Function
MOS SF-36 Medical Outcomes Study Short Form-36
MRM multiple reaction monitoring
PANAS Positive and Negative Affect Schedule
PDQ Psychosexual Daily Questionnaire
PHQ-9 Patient Health Questionnaire
SHBG sex hormone-binding globulin
TT total testosterone.

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