Association of Testosterone Levels With Anemia in Older Men: A Controlled Clinical Trial

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Study concept and design: Roy, Snyder, Artz, Bhasin, Cohen, Farrar, Cunningham, Matsumoto, Pahor, Ellenberg.

Acquisition, analysis, or interpretation of data: All authors.

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Abstract

Importance—In one-third of older men with anemia, no recognized cause can be found.

Objective—To determine if testosterone treatment of men 65 years or older with unequivocally low testosterone levels and unexplained anemia would increase their hemoglobin concentration.

Design, Setting, and Participants—A double-blinded, placebo-controlled trial with treatment allocation by minimization using 788 men 65 years or older who have average testosterone levels of less than 275 ng/dL. Of 788 participants, 126 were anemic (hemoglobin ≤12.7 g/dL), 62 of whom had no known cause. The trial was conducted in 12 academic medical centers in the United States from June 2010 to June 2014.
**Interventions**—Testosterone gel, the dose adjusted to maintain the testosterone levels normal for young men, or placebo gel for 12 months.

**Main Outcomes and Measures**—The percent of men with unexplained anemia whose hemoglobin levels increased by 1.0 g/dL or more in response to testosterone compared with placebo. The statistical analysis was intent-to-treat by a logistic mixed effects model adjusted for balancing factors.

**Results**—The men had a mean age of 74.8 years and body mass index (BMI) (calculated as weight in kilograms divided by height in meters squared) of 30.7; 84.9% were white. Testosterone treatment resulted in a greater percentage of men with unexplained anemia whose month 12 hemoglobin levels had increased by 1.0 g/dL or more over baseline (54%) than did placebo (15%) (adjusted OR, 31.5; 95% CI, 3.7-277.8; \( P = .002 \)) and a greater percentage of men who at month 12 were no longer anemic (58.3%) compared with placebo (22.2%) (adjusted OR, 17.0; 95% CI, 2.8-104.0; \( P = .002 \)). Testosterone treatment also resulted in a greater percentage of men with anemia of known cause whose month 12 hemoglobin levels had increased by 1.0 g/dL or more (52%) than did placebo (19%) (adjusted OR, 8.2; 95% CI, 2.1-31.9; \( P = .003 \)). Testosterone treatment resulted in a hemoglobin concentration of more than 17.5 g/dL in 6 men who had not been anemic at baseline.

**Conclusions and Relevance**—Among older men with low testosterone levels, testosterone treatment significantly increased the hemoglobin levels of those with unexplained anemia as well as those with anemia from known causes. These increases may be of clinical value, as suggested by the magnitude of the changes and the correction of anemia in most men, but the overall health benefits remain to be established. Measurement of testosterone levels might be considered in men 65 years or older who have unexplained anemia and symptoms of low testosterone levels.

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The prevalence of anemia is approximately 10 percent in older adults\(^1\) and tends to be higher in men than in women.\(^2\),\(^4\),\(^5\) Several causes of anemia in the elderly are recognized, including iron and vitamin B12 deficiencies, chronic inflammation and disease, chronic renal insufficiency, and the myelodysplastic syndromes. In approximately one-third of older adults with anemia, however, no recognized cause can be found.\(^1\),\(^6\)-\(^10\) No treatment has been shown to improve this unexplained anemia.

One possible cause of unexplained anemia in older men is testosterone deficiency, because serum testosterone levels decline as men age,\(^11\)-\(^13\) and testosterone treatment of men with very low testosterone increases their hemoglobin levels.\(^14\) In 2 population studies, low testosterone levels were significantly associated with unexplained anemia.\(^7\),\(^15\) Although testosterone treatment of older men with low testosterone levels has been shown to increase their hemoglobin concentrations,\(^16\)-\(^18\) few studies have specifically addressed the effect of testosterone in anemic men, and none has examined the effect of testosterone on men with unexplained anemia. The main goal of this study—The Anemia Trial of the Testosterone Trials—was to test the hypothesis that testosterone treatment of men aged 65 years or older with unequivocally low testosterone levels and unexplained anemia would increase their hemoglobin concentrations more than placebo.\(^19\)
Methods

Study Design

The Testosterone Trials are 7 coordinated trials of the efficacy of testosterone in older men who have low testosterone concentrations. They were conducted at 12 clinical trial sites in the United States.\textsuperscript{19} Participants were allocated to receive testosterone or placebo gel for 1 year, and efficacy was assessed every 3 months. This report describes the results of the Anemia Trial.

The protocol (Supplement 1) was approved by the institutional review boards of all participating institutions. All men gave written, informed consent to be included if they had low hemoglobin levels. A data safety monitoring board reviewed data unblinded every 3 months.

Participants

The main Testosterone Trials inclusion criteria were men ages 65 years or older, 2 serum testosterone level test results that averaged less than 275 ng/dL, and low risk of prostate cancer.\textsuperscript{19,20} Potential participants were excluded if their hemoglobin levels were less than 10.0 g/dL (to convert to g/L multiply by 10.0). Men whose baseline hemoglobin levels were 12.7 g/dL or lower were included in the Anemia Trial. This value identifies anemia with greater precision than WHO criteria.\textsuperscript{21} Further testing subclassified anemia into unexplained anemia and anemia of known cause. The primary analysis was performed in men with unexplained anemia, but analyses were also performed in men with anemia of known cause and nonanemic men.

Classification of men as anemic and of the type of anemia was made on blood drawn at baseline but not analyzed until the end of the trial. Classification of anemia of inflammation was adjudicated by case reviews by a panel of 3 hematologists blinded to outcome. Anemic participants were classified as having a known cause if they had a serum creatinine level of 2.2 mg/dL (to convert to μmol/L multiply by 76.25) or higher (renal insufficiency); either a mean corpuscular volume (MCV) of 105 fL or more and platelet count of 120 000/ μL (to convert to ×10\textsuperscript{9}/L multiply by 1) or less or an MCV of 105 fL or more and an absolute neutrophil count less than 1200/ μL (myelodysplasia); a ferritin level less than 40 ng/mL (iron deficiency) (to convert to pmol/L multiply by 2.247); folate levels less than 3.4 ng/mL (folate deficiency) (to convert to nmol/L multiply by 2.266); vitamin B\textsubscript{12} less than 200 pg/mL (B\textsubscript{12} deficiency) (to convert to pmol/L multiply by 0.7378); ferritin levels higher than 500 ng/mL and transferrin saturation less than 50% or ferritin levels higher than 40 ng/mL and a history of a medical condition or medication indicating chronic disease or inflammation (anemia of inflammation); haptoglobin levels less than 14 mg/dL (to convert to mg/L multiply by 10) and MCV greater than 100 fL (hemolytic anemia); and IgG, IgA or IgM levels higher than 1.0 g/dL (plasma cell dyscrasia and/or monoclonal gammopathy). Anemic participants who met none of these criteria were classified as having unexplained anemia.

Men were not treated for anemia as part of the trial, although some took iron outside of the trial, as follows: At baseline, unexplained anemia, 7 (testosterone/placebo 3/4); known
cause, 8 (testosterone/placebo 3/5). At month 12, unexplained anemia, 6 (testosterone/placebo 4/2); known cause, 8 (testosterone/placebo 5/3).

**Testosterone Treatment**

We allocated participants to treatment by minimization, which allows balancing with a larger number of variables than stratified randomization. Participants were assigned to the optimally balancing treatment with 80% probability. Balancing variables included age under or over 75 years and screening testosterone levels under or over 200 ng/dL.

Testosterone was administered as a 1% gel in a pump bottle (AndroGel; AbbVie). Placebo gel was similar. The dose was initially 5 g per day and was adjusted to attempt to keep the concentration at levels that are normal for young men. The adjustment was made by a staff member in the data coordinating center based on a prespecified algorithm. Measurements were made at a central laboratory (Quest Clinical Trials) at months 1, 2, 3, 6, and 9. To maintain blinding when the dose was adjusted in a man taking testosterone gel, the dose was changed simultaneously in a man taking placebo gel. No participant or site staff member was aware of the treatment assignment.

**Assessments**

Whole blood was drawn at baseline and at months 3, 6, 9, and 12 for measurement of hemoglobin levels at a central laboratory (Quest Clinical Trials). Red blood cell indices were determined on whole blood, and creatinine levels and total protein were measured on sera from baseline. At the end of the trial, the serum concentrations of ferritin, transferrin saturation, folate, B12, and haptoglobin were measured in baseline sera that had been stored at −80°C. Protein electrophoresis was performed when the total protein was more than 7.9 g/dL.

All participants in the Testosterone Trials were assessed for walking ability by the 6-minute Walk Test and for vitality by the Functional Assessment of Chronic Illness Therapy (FACIT)-Fatigue Scale at 0, 3, 6, 9, and 12 months. Patient Global Impression of Change (PGIC) questions about overall health, walking, sexual desire, energy, and memory were also asked every 3 months.

Serum concentrations of testosterone, free testosterone, and estradiol were measured in sera from blood drawn at baseline and months 3, 6, 9, and 12 and stored at −80°C. These assays were performed at the Brigham Research Assay Core.

**Statistical Analyses**

Participants were analyzed in the treatment group to which they were allocated, according to the intent-to-treat principle. The primary and secondary endpoints of dichotomous and continuous change in hemoglobin in the men with unexplained anemia were prespecified. A change of 1.0 g/dL or more for the dichotomous outcomes was selected, because this increase in hemoglobin concentration has previously been used to distinguish responders and nonresponders and has been associated with perceived improvement in quality of life. Additional prespecified exploratory analyses included assessment of the effect of
assignment to testosterone vs placebo treatment on hemoglobin levels in men with anemia of known cause and nonanemic men. Exploratory outcomes that were not prespecified were the association of changes in hemoglobin with other outcomes. Data were analyzed by generalized linear mixed models for longitudinal data for both assigned treatment and change in hemoglobin at each month, with logistic and linear models for dichotomous and continuous outcomes, respectively. Treatment effect heterogeneity across anemic groups was evaluated by the interaction of treatment with anemia classification. The PGIC results were analyzed as ordinal variables by mixed effects proportional odds models. All models included a random intercept for participant. Treatment arm, visit month, baseline hemoglobin levels, and balancing factors were fixed effects. For dichotomous outcomes, the treatment effect is represented by the odds ratio (OR) of the probability of a positive vs a negative response for participants who were assigned to testosterone vs placebo treatment, averaged over all months. For continuous outcomes the treatment effect is the model-based difference in the change from baseline for participants assigned to testosterone vs placebo, averaged over all months. For the association of change in hemoglobin with vitality and walking, models provide an estimate of the mean change in the outcome for a 1.0 g/dL increase in hemoglobin averaged over all months. These outcomes were continuous. All analyses were conducted at a 2-sided significance level of .05. No adjustments were made for multiple comparisons.

Sensitivity of the primary analysis to missing data was assessed by a worst-case-scenario analysis in which the model was rerun after all missing outcomes among placebo-treated subjects were set to 1, indicating an increase in hemoglobin levels of 1g/dL or more, and all missing outcomes among testosterone-treated subjects were set to 0, indicating a change in hemoglobin levels of less than 1g/dL.

Cumulative distribution functions of the average change in hemoglobin levels by treatment arm assessed the impact of testosterone on changes in hemoglobin levels other than 1.0 g/dL. Significance was assessed by the Kolmogorov-Smirnov test.

Sample size for the primary outcome, unexplained anemia, was not prespecified but was defined by the number of men in the Testosterone Trials who had a hemoglobin concentration of 12.7 g/dL or less and for whom no cause could be found. At the achieved minimum sample size of 27 patients per arm, there was 80% power to detect a difference in the percentage of men achieving a change in hemoglobin levels of at least 1g/dL if the true OR were 6.0 comparing testosterone-treated men with placebo-treated men, assuming the observed response rate of 10% among placebo-treated men, and conservatively assuming an intraclass correlation of $\rho = 1$ among repeated measurements in a participant. This corresponds to a response rate of 40% among testosterone-treated men.

### Results

#### Participants

Recruitment began in June 2010. Planned accrual was completed in June 2013. Treatment was completed in June 2014. Of the 788 men who enrolled in the Testosterone Trials, 126 were anemic at baseline with a hemoglobin level of 12.7 g/dL or less. Of these, 64 had
anemia of known cause: 8 (6.3%) owing to myelodysplasia, 42 (33.3%) iron deficiency, 3 
B₁₂ deficiency (2.4%), 10 chronic inflammation or disease (7.9%), and 1 plasma cell 
dyscrasia. The remaining 62 (49.2%) had unexplained anemia. Treatment assignment by 
category of anemia or not anemic is shown in Figure 1.

The characteristics at baseline of the participants are shown by treatment arm in Table 1. 
Race was somewhat imbalanced, but the few African Americans do not allow conclusions 
about race. Sex hormone binding globulin levels were imbalanced among nonanemic men, 
but these differences are not likely important.

Overall retention was 90.5%, similar in both treatment arms (Figure 1). The median serum 
testosterone concentration was below normal for young men prior to treatment and increased 
to mid-normal in both categories of anemic men and in the nonanemic men at 3, 6, 9, and 12 
months of testosterone treatment (eFigure 1 in Supplement 2). Testosterone concentrations 
in the placebo-treated men were lower than normal prior to treatment and did not change 
appreciably during treatment.

**Effect of Testosterone Treatment on Hemoglobin Concentration**

Testosterone treatment increased the percentage of men whose hemoglobin concentration 
Improved by 1.0 g/dL or more over the baseline value more than placebo treatment in men 
with unexplained anemia (AOR, 31.5; 95% CI, 3.7-277.8; \( P = .002 \) (Figure 2) (Table 2) 
(eFigure 2 in Supplement 2) (primary outcome). At month 12, 54% of testosterone-treated 
men but only 15% of placebo-treated men had experienced increases of 1.0 g/dL or more 
above baseline (AOR, 31.5; 95% CI, 3.7-277.8) (Table 2). A similar effect occurred in men 
with known causes of anemia (AOR, 8.2; 95% CI, 2.1-31.9; \( P = .003 \), and in nonanemic 
men (OR, 20.7; 95% CI, 12.9-33.3; \( P < .001 \)). the OR for an increase of 1.0 g/dL or more 
for testosterone-vs placebo-treated men did not differ by baseline anemia classification (\( P = .09 \)). The worst-case-scenario sensitivity analysis for missing data suggested a nearly 
significant greater percent of men in the testosterone arm whose hemoglobin increased by 
1.0 g/dL or more (OR, 5.0; 95% CI, 0.83-29.4; \( P = .07 \)).

Testosterone also increased hemoglobin concentrations by continuous analysis in men with 
unexplained anemia (secondary outcome) (adjusted mean difference [AMD], 0.83 g/dL; 
95% CI, 0.48-1.39; \( P < .001 \)) (eFigure 2 in Supplement 2), men with known causes of 
anemia (AMD, 0.64 g/dL; 95% CI, 0.12-1.17; \( P = .02 \), and nonanemic men (AMD, 0.90 
g/dL; 95% CI, 0.78-1.03; \( P < .001 \)). The effect of testosterone on continuous change in 
hemoglobin levels did not differ by anemia classification (\( P = .43 \)).

At month 12, 12 of 24 (58.3%) testosterone-treated men with unexplained anemia at 
baseline were no longer anemic, compared with 6 of 24 (22.2%) placebo-treated men (OR, 
17.0; 95% CI, 2.8-104.0; \( P = .002 \)). In 25 men with anemia of known cause at baseline and 
treated with testosterone, 15 (60%) were no longer anemic at month 12, compared with 4 of 
27 (14.8%) men treated with placebo (OR, 9.4; 95% CI, 1.4-60.5; \( P = .02 \)).

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Placebo-treated men had lower average hemoglobin level changes than testosterone-treated men, as shown by comparison of the cumulative distribution functions (eFigure 3 in Supplement 2).

**Increases in Hemoglobin Levels Related to Other Outcomes**

The increase in hemoglobin in response to testosterone in all anemic men, an exploratory outcome, was positively and significantly associated with very small increases in 6-minute walking distance and vitality, as assessed by the FACIT-Fatigue Scale. An increase in hemoglobin levels of 1.0 g/dL was associated with an increase of 8.3 meters in 6-minute walking distance ($P < .001$) and a 1.0 point improvement in the FACIT-Fatigue Scale ($P < .02$) in all anemic men (eFigure 4 in Supplement 2).

The increase in hemoglobin levels was also positively associated with Patient Global Impression of Change in all 5 areas tested, but the association reached statistical significance only for general health (AOR, 1.8; 95% CI, 1.3-2.5; $P = .001$) and energy (OR, 1.9; 95% CI, 1.2-3.0; $P = .008$), but not for walking ability (OR, 1.2; 95% CI, 0.8-1.7; $P = .33$), sexual desire (OR, 1.6; 95% CI, 0.9-2.8; $P = .13$), or memory (OR, 1.3; 95% CI, 0.9-1.8; $P = .17$) (Figure 3).

**Adverse Events**

Six of the 336 men who were not anemic at baseline and were treated with testosterone developed hemoglobin values of 17.5 g/dL or higher during treatment (eTable in Supplement 2). Four of the 6 had serum testosterone concentrations higher than 800 ng/dL when erythrocytosis was detected. In all Testosterone Trials participants, there were no significant differences between treatment arms in major adverse cardiovascular events, serious adverse events, or prostate cancer.22

**Discussion**

Testosterone treatment of these men who also had unexplained anemia significantly increased the percentage of those whose hemoglobin concentration improved by 1.0 g/dL or more above baseline compared with placebo treatment. Among men with unexplained anemia, 58.3% of those treated with testosterone were no longer anemic at month 12, compared with 22.2% treated with placebo. Testosterone treatment also resulted in similar improvements in men who were anemic for a known cause and in men who were not anemic. Testosterone treatment of men ages 65 years or older who had a mean testosterone level of less than 275 ng/dL resulted in improvement in all aspects of sexual function and some benefit in mood and depressive symptoms but no benefit in vitality.22 In all anemic men combined, there were small but statistically significant positive associations between the increases in hemoglobin levels and improvement in walking and vitality. Increases in hemoglobin levels were positively and significantly associated with participants' global impression of change in overall health and energy.

The overall incidence of any anemia in all men enrolled in the Testosterone Trials was 16.0%, which is higher than the approximately 10% to 15% reported in some surveys of anemia in older men1,26,27 but lower than the approximately 24% reported in another.28
perhaps representing differences in demographics or illnesses among the different populations. Among the anemic men in the Testosterone Trials, the incidence of unexplained anemia was 49.2%, similar to that in 2 other studies. In a study of 69 anemic men, 53.6% had unexplained anemia.9 In a study of 190 anemic patients, of whom 85% were men, 35% had unexplained anemia.10

Although the effect of testosterone on older men with unexplained anemia has not been reported previously, the stimulatory effect of testosterone on hemoglobin levels in men with low testosterone is well established. Testosterone has been shown to increase hemoglobin levels in men with low-normal testosterone owing to no discernable reason other than age29 and in men with markedly low testosterone owing to pituitary or testicular disease.14 This effect of testosterone is dose-dependent, more so in older than in younger men.30 In 33 older men with low testosterone and anemia, testosterone treatment corrected the anemia in 21.31

The finding that testosterone treatment of men 65 years or older with low testosterone increased hemoglobin concentration whether they were anemic or not confirms that testosterone stimulates erythropoiesis in hypogonadal men. The improvement in hemoglobin levels by physiologic replacement of testosterone in men who had known causes of anemia suggests that their anemia was owing to low testosterone as well as to the known cause, such as iron deficiency.

The finding that testosterone treatment of men 65 years or older with low testosterone and unexplained anemia increased hemoglobin concentration by more than 1.0 g/dL is important; previously no treatment has been reported to improve unexplained anemia in older men. These results are also important because they suggest that evaluation of unexplained anemia in older men with symptoms consistent with hypogonadism might include measurement of serum testosterone. However, the percentage of older men with unexplained anemia who have low testosterone compared with the percentage in all older men is not known.

Although the increase in hemoglobin levels was significantly associated with improvements in walking distance and vitality, the degrees of improvement were modest. An increase in hemoglobin of 1.0 g/dL was associated with an increase in walking distance of 8.3 meters, whereas an increase of 50 meters has been shown to be the minimum distance at which an individual can detect improvement in walking.32 An increase in hemoglobin levels of 1.0 g/dL was associated with an improvement of 1 point in the FACIT-Fatigue Scale of vitality, whereas a 3 to 4 point increase would be needed to convey clinical meaningfulness.33 Nonetheless, greater increases in hemoglobin levels were significantly associated with the participants’ global impression of change in overall health and energy, suggesting that the increase in hemoglobin levels was clinically significant.

Testosterone treatment resulted in erythrocytosis only in nonanemic men, and only in 6 (2%) of 336 participants, mostly when the serum testosterone was elevated. This finding supports the recommendation to monitor hemoglobin levels during testosterone treatment.34
Limitations

The Anemia Trial had several strengths, including a placebo-controlled, double-blind design, enrollment of men who had unequivocally low testosterone and hemoglobin levels by stringent criteria, and excellent participant retention. A major limitation of this trial, although by design, is that the results apply only to men 65 years or older who have low testosterone concentrations. They do not apply to men with unexplained anemia but normal testosterone concentrations. Another limitation is the small sample size for each anemia subgroup, resulting in wide confidence intervals for the estimated treatment effects. Lack of a prespecified adjustment for multiple comparisons resulted in an inflated probability of a false-positive result, but posthoc review suggested that the strongly significant findings for changes in hemoglobin levels across anemic subgroups still holds after Holm-Bonferroni adjustment.35

Conclusions

Among older men with low testosterone, testosterone treatment significantly increased hemoglobin levels in those with unexplained anemia and those with anemia associated with known causes. These increases may be of clinical significance, as suggested by the magnitude of the increases and the correction of anemia in the majority of men. The overall health benefits, however, remain to be determined. These results also suggest that measurement of serum testosterone levels might be considered in men 65 years or older who have unexplained anemia and symptoms of hypogonadism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References


**Key Points**

**Question** Will testosterone treatment of older men with low testosterone levels and mild anemia improve their anemia?

**Findings** Testosterone treatment of older men with low testosterone levels and unexplained anemia corrected the anemia more than placebo. This treatment also corrected anemia more than placebo in men who had anemia of known causes, such as iron deficiency.

**Meaning** Testosterone deficiency in older men results in decreased hemoglobin levels and sometimes in mild anemia. Correcting the testosterone deficiency is associated with increased hemoglobin levels and tends to correct the anemia, even in the presence of a coexisting cause of anemia.
Figure 1. Screening and Retention of Participants in the Anemia Trial

a Five Testosterone Trials participants were missing baseline hemoglobin levels and were not classified with respect to anemia. An additional 2 were randomized incorrectly and did not provide any baseline clinical data.

b Participants excluded from analysis and with no follow-up data are the same.
Figure 2. Association of Testosterone vs Placebo Treatment for 12 Months With Hemoglobin Concentrations in Participants in the Anemia Trial

A and B, Results in men with unexplained anemia. C and D, Results in men with anemia of known causes. E and F, Results in nonanemic men. The graphs on the left (A, C, and E) show the results as dichotomous variables, ie, the percent of men who demonstrated an increase above baseline of 1.0 g/dL or more, and the graphs on the right (B, D, and F) show the results continuously. The values are means ± pointwise confidence intervals.
Figure 3. Relationship Between Change in Hemoglobin Levels and Patient Global Impression of Change Questions in All Anemic Men in the Testosterone Trials

Participants were asked at each 3-month visit if their overall health, walking, sexual desire, energy and memory were much better, a little better, not changed, a little worse, or a lot worse than at the beginning of the trial. For analysis, the much worse and little worse categories were combined. Boxes represent the interquartile ranges (IQR), and the whiskers represent 1.5× the IQR for the change in hemoglobin levels pooled across months. *P* values were calculated by a mixed effects proportional odds model with 4-level PGIC response as
the outcome and change in hemoglobin levels as the primary predictor. Models adjusted for treatment arm and balancing factors age, as described in the Methods section.
## Table 1

Characteristics of Participants in the Anemia Trial at Baseline

<table>
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<tr>
<td>Age, mean (SD)</td>
<td></td>
<td>75.6 (7.5)</td>
<td>74.8 (6.0)</td>
<td>74.9 (6.3)</td>
<td>73.7 (4.9)</td>
<td>71.7 (5.7)</td>
<td>71.7 (5.3)</td>
</tr>
<tr>
<td>Race, No. (%)^a</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>White</td>
<td></td>
<td>29 (82.9)</td>
<td>25 (92.6)</td>
<td>28 (80.0)</td>
<td>25 (86.2)</td>
<td>296 (88.1)</td>
<td>291 (90.7)</td>
</tr>
<tr>
<td>African American</td>
<td></td>
<td>5 (14.3)</td>
<td>0 (0)</td>
<td>3 (8.6)</td>
<td>3 (10.3)</td>
<td>18 (5.4)</td>
<td>12 (3.7)</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>1 (2.9)</td>
<td>2 (7.4)</td>
<td>4 (11.4)</td>
<td>1 (3.4)</td>
<td>22 (6.5)</td>
<td>18 (5.6)</td>
</tr>
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<td>Ethnicity, No. (%)</td>
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<tr>
<td>Hispanic</td>
<td></td>
<td>0 (0)</td>
<td>2 (7.4)</td>
<td>1 (2.9)</td>
<td>2 (6.9)</td>
<td>14 (4.2)</td>
<td>9 (2.8)</td>
</tr>
<tr>
<td>Non-Hispanic</td>
<td></td>
<td>35 (100)</td>
<td>25 (92.6)</td>
<td>34 (97.1)</td>
<td>27 (93.1)</td>
<td>321 (95.5)</td>
<td>312 (97.2)</td>
</tr>
<tr>
<td>College graduate</td>
<td></td>
<td>21 (60.0)</td>
<td>17 (63.0)</td>
<td>11 (31.4)</td>
<td>14 (48.4)</td>
<td>181 (53.9)</td>
<td>163 (50.8)</td>
</tr>
<tr>
<td>Married/partner</td>
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<td>23 (65.7)</td>
<td>23 (85.2)</td>
<td>25 (71.4)</td>
<td>18 (62.1)</td>
<td>247 (73.5)</td>
<td>253 (78.8)</td>
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<tr>
<td>Concomitant conditions, mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>BMI</td>
<td></td>
<td>29.6 (3.9)</td>
<td>31.7 (4.0)</td>
<td>30.9 (3.6)</td>
<td>30.9 (3.1)</td>
<td>30.9 (3.5)</td>
<td>31.2 (3.5)</td>
</tr>
<tr>
<td>BMI&gt;30 (%)</td>
<td></td>
<td>19 (54.3)</td>
<td>17 (63.0)</td>
<td>20 (57.1)</td>
<td>18 (62.1)</td>
<td>214 (63.7)</td>
<td>205 (63.9)</td>
</tr>
<tr>
<td>Alcohol (drinks/wk)</td>
<td></td>
<td>2.1 (3.3)</td>
<td>2.6 (4.1)</td>
<td>2.7 (4.7)</td>
<td>3.8 (5.3)</td>
<td>3.0 (4.2)</td>
<td>3.7 (5.2)</td>
</tr>
<tr>
<td>Smoking, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Current (%)</td>
<td></td>
<td>2 (5.7)</td>
<td>0 (0)</td>
<td>3 (8.6)</td>
<td>3 (10.3)</td>
<td>27 (8.0)</td>
<td>28 (8.7)</td>
</tr>
<tr>
<td>Ever (%)</td>
<td></td>
<td>21 (60.0)</td>
<td>20 (74.0)</td>
<td>25 (71.4)</td>
<td>18 (62.1)</td>
<td>216 (64.3)</td>
<td>220 (68.5)</td>
</tr>
<tr>
<td>Diabetes, No. (%)</td>
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<td>13 (37.1)</td>
<td>16 (59.3)</td>
<td>18 (51.4)</td>
<td>20 (69)</td>
<td>112 (33.3)</td>
<td>111 (34.6)</td>
</tr>
<tr>
<td>Hypertension, No. (%)</td>
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<td>26 (74.3)</td>
<td>23 (85.2)</td>
<td>28 (80)</td>
<td>27 (93.1)</td>
<td>235 (69.9)</td>
<td>225 (70.1)</td>
</tr>
<tr>
<td>MI, No. (%)</td>
<td></td>
<td>8 (22.9)</td>
<td>4 (14.8)</td>
<td>4 (11.4)</td>
<td>1 (3.4)</td>
<td>48 (14.3)</td>
<td>50 (15.6)</td>
</tr>
<tr>
<td>Stroke, No. (%)</td>
<td></td>
<td>1 (2.9)</td>
<td>1 (3.7)</td>
<td>5 (14.3)</td>
<td>1 (3.4)</td>
<td>14 (4.2)</td>
<td>11 (3.4)</td>
</tr>
<tr>
<td>Sleep apnea, No. (%)</td>
<td></td>
<td>4 (11.4)</td>
<td>9 (33.3)</td>
<td>6 (17.1)</td>
<td>5 (17.2)</td>
<td>62 (18.5)</td>
<td>66 (20.6)</td>
</tr>
<tr>
<td>Hemoglobin levels, mean (SD), g/dL</td>
<td></td>
<td>12.1 (0.6)</td>
<td>11.9 (0.7)</td>
<td>11.7 (0.9)</td>
<td>12.1 (0.4)</td>
<td>14.3 (0.9)</td>
<td>14.4 (0.9)</td>
</tr>
</tbody>
</table>

^a Numbers may not sum to 100% because of missing data.
<table>
<thead>
<tr>
<th>Characteristic Treatment</th>
<th>Unexplained Anemia</th>
<th>Anemia of Known Cause</th>
<th>Not Anemic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Testosterone</td>
<td>Placebo</td>
</tr>
<tr>
<td>Sex hormones, mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone, ng/dL</td>
<td>221.2 (77.2)</td>
<td>201.6 (71.6)</td>
<td>222.7 (68.2)</td>
</tr>
<tr>
<td>Free T, pg/mL</td>
<td>52.0 (21.7)</td>
<td>52.6 (23.5)</td>
<td>57.8 (19.7)</td>
</tr>
<tr>
<td>DHT, ng/dL</td>
<td>18.7 (13.4)</td>
<td>18.9 (10.2)</td>
<td>17.1 (7.5)</td>
</tr>
<tr>
<td>Estradiol, pg/mL</td>
<td>17.8 (5.7)</td>
<td>17.9 (6.2)</td>
<td>17.3 (5.5)</td>
</tr>
<tr>
<td>SHBG, nM</td>
<td>38.5 (18.2)</td>
<td>34.8 (19.5)</td>
<td>34.7 (20.6)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); DHT, dihydrotestosterone; MI, myocardial infarction; SHBG, sex hormone binding globulin.

*In men with unexplained anemia, the testosterone and placebo groups differed with regard to race (*P* = .05).  

*In nonanemic men, the testosterone and placebo groups differed (*P* = .01).  with regard to sex hormone binding globulin.
**Table 2**

Effect of Testosterone on Hemoglobin Levels

<table>
<thead>
<tr>
<th>Participant Group</th>
<th>Outcome</th>
<th>Treatment</th>
<th>No.</th>
<th>Baseline, Mean (SD)$^a$</th>
<th>Change From Baseline Values, Months$^d$</th>
<th>Effect (95% CI)</th>
<th>Size$^c$</th>
<th>P Value$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unexplained anemia</td>
<td>Hemoglobin (dichotomous)$^e$</td>
<td>Testosterone</td>
<td>27</td>
<td>11.9 (0.7)</td>
<td>6/24 (25)</td>
<td>13/24 - 31.5 (3.7-277.8)</td>
<td>.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>35</td>
<td>12.1 (0.6)</td>
<td>3/27 (11)</td>
<td>2/28 (7) - 4/27 (15)</td>
<td>.163</td>
<td></td>
</tr>
<tr>
<td>Primary outcome</td>
<td>Hemoglobin (continuous)</td>
<td>Testosterone</td>
<td>27</td>
<td>11.9 (0.7)</td>
<td>0.6 (1.1)</td>
<td>.83 (0.48-1.39) - 1.30 (0.75-2.18)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>35</td>
<td>12.1 (0.6)</td>
<td>0.1 (0.8)</td>
<td>0.2 (0.6) - 0.2 (0.8)</td>
<td>.002</td>
<td></td>
</tr>
<tr>
<td>Secondary outcome</td>
<td>Hemoglobin (continuous)</td>
<td>Testosterone</td>
<td>27</td>
<td>11.9 (0.7)</td>
<td>0.6 (1.1)</td>
<td>.83 (0.48-1.39) - 1.30 (0.75-2.18)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>35</td>
<td>12.1 (0.6)</td>
<td>0.1 (0.8)</td>
<td>0.2 (0.6) - 0.2 (0.8)</td>
<td>.002</td>
<td></td>
</tr>
<tr>
<td>Exploratory outcomes</td>
<td>Anemia of known cause</td>
<td>Hemoglobin (dichotomous)$^e$</td>
<td>29</td>
<td>12.1 (0.4)</td>
<td>0.6 (1.2)</td>
<td>0.9 (1.2) - 0.9 (1.1)</td>
<td>0.64 (0.12-1.17) - 0.90 (0.17-1.65)</td>
<td>.018</td>
</tr>
<tr>
<td></td>
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<td>Placebo</td>
<td>35</td>
<td>11.7 (0.9)</td>
<td>-0.1 (0.7)</td>
<td>0.1 (0.6) - 0.1 (0.9)</td>
<td>0.3 (0.9)</td>
<td></td>
</tr>
<tr>
<td>No anemia</td>
<td>Hemoglobin (dichotomous)$^e$</td>
<td>Testosterone</td>
<td>336</td>
<td>14.3 (0.9)</td>
<td>0.5 (0.9)</td>
<td>0.6 (1.1) - 0.6 (1.1)</td>
<td>0.9 (0.78-1.03) - 0.99 (0.86-1.13)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>321</td>
<td>14.4 (0.9)</td>
<td>-0.3 (0.7)</td>
<td>-0.3 (0.8) - 0.3 (0.8)</td>
<td>-0.4 (0.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hemoglobin (continuous)</td>
<td>Testosterone</td>
<td>336</td>
<td>14.3 (0.9)</td>
<td>0.5 (0.9)</td>
<td>0.6 (1.1) - 0.6 (1.1)</td>
<td>0.9 (0.78-1.03) - 0.99 (0.86-1.13)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>321</td>
<td>14.4 (0.9)</td>
<td>-0.3 (0.7)</td>
<td>-0.3 (0.8) - 0.3 (0.8)</td>
<td>-0.4 (0.9)</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Values are means (SDs) for continuous outcomes and No. (%) for dichotomous outcomes.

$^b$The treatment effect for continuous outcomes is the mean difference in change from baseline for participants allocated to testosterone vs placebo, adjusted for balancing factors: baseline total testosterone (≤200ng/dL), age (≤75 years), site, participation in the main trials, use of antidepressants, and use of PDE-5 inhibitors.

$^c$The effect size is the treatment effect divided by the baseline standard deviation.

$^d$The $P$ value for the significance of the treatment effect was determined by a linear mixed model for continuous outcomes and logistic mixed model for dichotomous outcomes with a random intercept for participant.

$^e$Dichotomous hemoglobin response is an increase of 1 g/dL or more from baseline.