Testosterone supplementation (TS) is assumed important for cognitive functioning in men, but conflicting results have prevented firm conclusions. The current study systematically reviewed available randomized controlled trials (RCTs) on effects of TS on cognitive functioning in men, subjected the findings to meta-analysis, and explored between-study differences as possible moderators of the effects. Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines, two authors independently searched for eligible records in the electronic databases of PubMed, PsycINFO, Web of Science, the Cochrane Library, Cumulative Index of Nursing and Allied Health, and Embase and determined eligibility using the following (population, intervention, comparison, outcome) criteria: population, male adults (>18 years); intervention, TS; comparison, placebo; and outcome, results of standardized neuropsychological tests. Following duplicate removal, 3873 records were screened with 92 remaining for full-text screening. Twenty-one papers reporting results of 23 independent RCTs were included, of which none treated samples of clinically hypogonadal men. The small improvement found in overall cognitive functioning ($Hedges' g = 0.09; CI 95%: -0.02 to 0.19$) failed to reach statistical significance ($P = 0.108$) and approached zero when adjusting for possible publication bias ($g = 0.04$). The effects for the 11 individual cognitive domains did not reach statistical significance ($g$: $-0.04$ to $0.19$, $P$: $0.061$ to $0.989$). Small statistically significant ($P < 0.05$) effects were found for five study subsets but failed to meet the fail-safe criterion. The available evidence indicates that effects of TS on cognitive functioning in men with testosterone levels within normal ranges are less robust and of insufficient magnitude to be of clinical relevance. The effects in clinically hypogonadal men remain to be investigated.

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Freeform/Key Words: testosterone supplementation, androgens, cognitive functions, systematic review, meta-analysis

From 2001 to 2011, global testosterone prescription sales increased 12 fold from $150 million to $1.8 billion [1], and this increase is expected to continue and reach $3.8 billion by 2022 [2]. One reason for this boom in prescription sales is an increased recognition of hypogonadism [3], a syndrome affecting an estimated 25% of men over age 65 [4] and characterized by low physiological testosterone together with clinical symptoms of hypogonadism, such as decreased libido, impaired erectile function, and decreased energy [5].

Abbreviations: AAMI, age-associated memory impairment; RCT, randomized controlled trial; TS, testosterone supplementation.
Physiological testosterone is hypothesized to be important for cognition in men, and this has been supported by several lines of research. First, testosterone appears to influence neurobiological processes associated with cognitive aging and the development of neurodegenerative disorders, such as Alzheimer’s disease. Testosterone has thus been found to delay neuronal apoptosis [6], accelerate the rate of nerve regeneration [7], modulate neuronal damage caused by oxidative stress [8], exert anti-inflammatory actions [9], and reduce beta amyloid peptide levels [10]. Second, cognitive functions deteriorate with advanced age [11], in parallel with an age-mediated decline in male testosterone levels, starting in the third decade [12]. Third, there is some evidence to suggest that prostate cancer patients receiving androgen-deprivation therapy are at increased risk of cognitive impairment and dementia compared with prostate cancer patients receiving other types of treatment [13, 14]. On this background, testosterone supplementation (TS) may possibly improve cognitive functioning in men.

Several trials have investigated the effect of TS on cognitive functioning in men, and whereas one previous systematic review [15] concluded that promising associations had been found between TS and cognitive functions in men with both normal and low levels of testosterone and in men with and without cognitive impairment, another systematic review [16] concluded that the use of TS to improve cognitive functioning was not supported by data from clinical trials. More recently, two nonsystematic reviews [17, 18] concluded that evidence indicates that TS has positive effects on cognitive functions, particularly in men with cognitive impairment and low testosterone levels. The conflicting conclusions from existing reviews reflect that the results of the existing trials vary considerably, which may possibly be a result of between-study differences in the included neuropsychological tests, as well as treatment modalities, e.g., dosage, duration, type, and route of administration [15]. As all of the available systematic and nonsystematic reviews [15–20] to date have been nonquantitative, narrative reviews, a need for a systematic review with quantitative meta-analysis is indicated. Our aim was therefore to conduct a systematic review and meta-analysis to evaluate the effect of TS on cognitive functioning in men and to explore possible moderating effects of between-study differences in relevant study characteristics. Our primary hypothesis was that TS would have a positive effect on overall cognitive functioning, i.e., the individual cognitive domains aggregated into one combined estimate of cognitive functioning. Furthermore, as existing findings indicate that men tend to outperform women on tasks that use visuospatial skills [21, 22] and that women’s visuospatial skills improve when they receive TS [23], we expected to find the strongest effects for cognitive domains that use these skills (i.e., visuospatial function, visuospatial learning, visuospatial memory, and visuomotor function). Finally, as a result of the suggested neuroprotective effect of testosterone against Alzheimer’s disease pathology [7, 10], we hypothesized that the effect of TS on cognitive functioning would be stronger in studies administering testosterone to cognitively impaired men, e.g., men with neurodegenerative disorders, such as Alzheimer’s disease.

1. Methods

A. Search Strategy and Selection Criteria

Two authors (C.R.B. and H.R.D.) independently searched for reports on the effect of TS on cognitive functions in men in the electronic databases of PubMed, PsycINFO, Web of Science, the Cochrane Library, Cumulative Index of Nursing and Allied Health, and Embase. The final searches were repeated and updated on 6 December 2018. The search strategy included key words for men, testosterone, cognitive functions, and their synonyms (search strategy and results can be obtained by request from the corresponding author). In addition, a backward search (snowballing) of reference lists of identified articles and earlier systematic reviews was conducted, together with a forward search (citation tracking). Only English-language publications in peer-reviewed journals were included. Eligibility was determined using the population, intervention, comparison, outcome approach [24]: population, a male adult (>18 years) healthy or clinical sample; intervention, TS; comparison, placebo; and
outcome, standardized neuropsychological test results. The two authors independently screened the identified papers, excluded noneligible studies, retrieved and evaluated full texts of the remaining papers, excluded studies with registration of reasons, and extracted \textit{a priori} specified data from eligible studies. The current study was preregistered with PROSPERO [25] (Number CRD42017060530) and is reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses recommendations [26].

\textbf{B. Data Analysis}

Hedges' $g$, which corrects for possible bias as a result of a small sample size [27], was chosen as the effect size. The data were combined using random-effects models. The effect of TS on overall cognitive functioning was chosen as the primary outcome and was calculated by pooling the effects for all cognitive domains across included studies. If a paper reported results of more than one neuropsychological test assessing the same cognitive domain, then a pooled, weighted effect size for that domain was calculated for the study in question to ensure independence. Secondary outcomes included pooled effect sizes calculated separately for individual cognitive domains reported in more than three studies. Effect sizes were categorized as small (0.2), medium (0.5), or large (0.8), respectively [28].

Studies were rated as having high, low, or unclear risk of bias using the Cochrane Collaborations tool for assessing risk of bias [29]. Industry sponsorship of pharmacotherapy trials has been associated with more favorable outcomes [30], and industry-sponsored studies were therefore rated as having a high risk of bias in the category of “other bias.” To evaluate incomplete outcome reporting, records of trial registrations were reviewed and authors contacted to clarify study characteristics when needed.

Statistical heterogeneity was evaluated using $I^2$, with values of 0%, 25%, 50%, and 75% taken to indicate no, low, moderate, and high heterogeneity, respectively [31]. The Q statistic was used to evaluate the probability that results reflect systematic between-study differences [32]. As a result of the generally low statistical power of heterogeneity tests [33], $P < 0.10$ was taken to indicate heterogeneity.

Positive and negative findings may not be equally likely to get published, thereby introducing risk of publication bias. The distribution of effect sizes was visually inspected by means of funnel plots and tested with Egger test [34]. If results were suggestive of publication bias, an adjusted effect size was estimated using Duval and Tweedie trim and fill method [35]. The file-drawer problem, \textit{i.e.}, the possibility that unidentified or unpublished studies with null findings could alter statistically significant meta-analysis results, was evaluated using Rosenthal fail-safe $n$ [36]. If the fail-safe number exceeded the criterion of $5K + 10$, with $K$ being the number of studies included in the meta-analysis, then the results were considered relatively robust in case of unpublished null findings.

Two types of sensitivity analyses were conducted. First, the meta-analyses were repeated with “0” imputed as the effect size when authors of included studies stated that they had obtained nonsignificant results for neuropsychological outcomes without presenting the data. Second, the meta-analyses were repeated with “winsorizing”, \textit{i.e.}, by truncating outliers so that they did not differ >2 SD from the original pooled effect size [37].

When available in a sufficient number of studies ($K > 8$), a number of categorical and continuous moderators were explored with unadjusted meta-regression analyses (random-effects model, maximum-likelihood method) [38]. These included treatment characteristics [TS type, duration, testosterone measurement characteristics (time of day, assay type), effect of TS on physiological testosterone levels from pre- to post-treatment in the intervention group compared with placebo (Hedges' $g$), participant characteristics (mean sample age, cognitive status, gonadal status), and study characteristics (publication year, industry sponsoring).

When associations between the included moderators reached statistical significance ($P < 0.05$) or were of medium magnitude ($r > 0.30$), moderators were analyzed together in adjusted models [27], taking the variance inflation factor into consideration [39, 40]. Between-study differences were further explored by pooling effect sizes for subgroups of studies according to the categorical moderators when available in at least two studies.
Comprehensive Meta-Analysis, version 3.3 [41], and IBM SPSS statistics, version 25 [42], were used for all analyses.

2. Results

The study selection process is shown in Fig. 1. Following duplicate removal, a total of 3873 records were screened with the two authors agreeing on 98.5% of the inclusion/exclusion decisions. After solving disagreement by negotiation, 92 records remained for full-text screening. The authors agreed on 82.4% of decisions. After solving disagreement by negotiation, a total of 71 records were excluded (data not shown; can be obtained by request from the corresponding author), resulting in 21 papers. One paper [43] presented separate data on Alzheimer’s patients and healthy men, and another [44] reported separate data on young and old men, yielding a total number of 23 independent randomized controlled trials (RCTs) to be included and subjected to meta-analysis. The characteristics of the included studies are shown in Table 1. The mean sample age was 64.9 years (SD = 13.0), and mean treatment duration was 33.4 weeks (SD = 42.1). The neuropsychological tests used in the 23 RCTs corresponded to 11 distinct cognitive domains (data not shown; can be obtained by request from the corresponding author). Seventeen studies included men who had testosterone levels within the normal range (mean total testosterone between 321 and 865 ng/dL),
| Study                | Cognitive Outcome                                                                 | n<sup>a</sup> | n<sup>b</sup> | Duration<sup>c</sup> | Type<sup>d</sup> | Doses     | Mean Age (Range/SD)<sup>e</sup> | Cognitive Status | Gonadal Status Pre<sup>f</sup> | Gonadal Status Post<sup>f</sup> | TS Effect<sup>g</sup> | T Medium | Assay          | Time          |
|---------------------|-----------------------------------------------------------------------------------|---------------|---------------|----------------------|-----------------|-----------|-------------------------------|------------------|--------------------------|---------------------|-----------------|------------|----------------|---------------|
| Janowsky et al.     | Executive function; verbal memory; visuomotor function                            | 56            | 56            | 12.00                | Patch           | 15 mg/d   | 67.40 (60–75)                 | Normal           | Normal 555.2 (132.6) pM | High 850.2 (397.3) pM | 1.11            | Serum     | Direct RIA    | Morning/       |
|                     | Attention/working memory                                                          | 19            | 19            | 4.00                 | Injection       | 150 mg/wk | 67.45 (61–75)                 | Normal           | Normal 423.3 (128.7) pM | High 1566.4 (1086.1) pM | 0.12            | Serum     | Direct RIA    | Morning/       |
| Cherrier et al.     | Executive function; language; verbal memory; visuospatial memory; attention/working memory | 28            | 25            | 6.00                 | Injection       | 100 mg/wk | 67.40 (50–80)                 | Normal           | Normal 576.4 (66.9) ng/dL | High 1239.2 (53.0) ng/dL | 7.68            | Serum     | Direct RIA    | Random/ not fasting |
| O'Connor et al.     | Executive function; language; verbal memory; visuomotor function; attention/working memory | 30            | 29            | 8.00                 | Injection       | 200 mg/wk | 28.30 (19–45)                 | Normal           | Normal 625.4 (66.9) ng/dL | High 1107.6 (111.2) ng/dL | 2.43            | Serum     | Direct CIA    | Random/ not fasting |
| Kenny et al.        | Executive function; language; attention/working memory                            | 67            | 44            | 32.00                | Patch           | 2–2.5 mg/d| 76.00 (65–87)                 | Normal           | Normal 91.0 (34.0) ng/dL  | Normal 162.0 (100) ng/dL  | 1.00            | Serum     | Direct RIA    | Morning/ fasting |
| Kenny et al.        | Cognitive status; executive function; visuomotor function; attention/working memory | 11            | 11            | 12.00                | Injection       | 200 mg/ 2 wk | 80.00 (73–87) | Impaired (MCI) | Normal 410.0 (112.0) ng/dL | High 1211.0 (600.0) ng/dL | 2.59            | Serum     | Direct RIA    | NR/ fasting    |
| Cherrier et al.     | Executive function; language; visuospatial memory; verbal memory; attention/working memory | 41            | 38            | 6.00                 | Injection       | 100 mg/wk | 65.00 (50–85)                 | Normal           | Normal 403.5 (230.6) ng/dL | High 1476.5 (734.9) ng/dL | 1.73            | Serum     | Direct CIA    | Random/ not fasting |
| Cherrier et al.     | Executive function; language; verbal memory; visuospatial memory; verbal learning; visuomotor function | 32            | 28            | 6.00                 | Injection       | 100 mg/wk | 76.00 (63–85)                 | Impaired (AD + MCI) | Normal 403.5 (72.9) ng/dL | High 1441.9 (230.6) ng/dL | 5.11            | Serum     | Direct CIA    | Random/ not fasting |
| Haren et al.        | Cognitive status; visuosensor function; attention/working memory                  | 76            | 58            | 32.00                | Pellets         | 80 mg/d   | 68.50 (60–86)                 | Normal           | Normal 482.4 (130.6) ng/dL | Normal 449.3 (236.0) ng/dL | 0.02            | Serum     | Direct CIA    | Morning/       |
| Lu et al.           | Cognitive status; verbal memory; visuomotor function; visuospatial function       | 20            | 14            | 24.00                | Gel (1%)        | 75 mg/d   | 69.80 (8.7)                  | Impaired (AD)    | Normal 387.7 (76.6) ng/dL | Normal 597.1 (554.3) ng/dL | 0.64            | Serum     | Direct RIA    | NR/HR        |
| Lu et al.           | Cognitive status; verbal memory; visuomotor function; visuospatial function       | 29            | 20            | 24.00                | Gel (1%)        | 75 mg/d   | 62.36 (6.7)                  | Normal           | Normal 385.8 (70.1) ng/dL | Normal 737.5 (241.9) ng/dL | 1.77            | Serum     | Direct RIA    | NR/HR        |

(Continued)
| Study              | Cognitive Outcome                                                                 | n<sup>a</sup> | n<sup>b</sup> | Duration<sup>c</sup> | Type<sup>d</sup> | Doses             | Mean Age (Range/SD)<sup>e</sup> | Cognitive Status       | Gonadal Status Pre<sup>f</sup> | Gonadal Status Post<sup>f</sup> | TS Effect<sup>g</sup> | T Medium | Assay                 | Time          |
|-------------------|-----------------------------------------------------------------------------------|---------------|---------------|----------------------|-----------------|----------------------|-------------------------------|--------------------------|--------------------------|--------------------------|-------------------|-----------|-----------------------|---------------|
| Cherrier et al.   | Verbal memory; visuospatial memory                                               | 57            | 50            | 6.00                 | Injection       | 50, 100, or 300 mg/6 wk | 67.00 (56–78) Normal             | Normal 386.3 (160.5) ng/dL | High 1911.5 (123.8) ng/dL | 2.50       | Serum                | Direct CIA    | Random/ not fasting |
| Maki et al.       | Executive function; verbal memory; verbal learning; visuospatial learning; attention/working memory | 15            | 15            | 12.90                | Injection       | 200 mg/2 wk            | 73.90 (66–86) Normal             | Normal 10.2 ± 3.2 pg/mL | High 970.21 (539.1) ng/dL | 1.82       | Serum                | Direct RIA and CIA | NR/NR                |
| Vaughan et al.    | Executive function; verbal memory; verbal learning; visuospatial learning; attention/working memory | 47            | 32            | 156.00               | Injection       | 200 mg/2 wk            | 70.80 (65–83) Normal             | Low-normal 285.3 (64.1) ng/dL | Normal 587.9 (279.9) ng/dL | 1.10       | Serum                | NR          | Morning/ NR         |
| Emmelot-Vonk et al. | Executive function; verbal memory; verbal learning; visuospatial function; attention/working memory | 237           | 223           | 26.00                | Pellets         | 160 mg/d              | 67.25 (60–80) Normal             | Low-normal 317.0 (54.8) ng/dL | Low-normal “unchanged” | −0.36      | Serum                | Direct CIA    | Morning/ fasting    |
| Young et al. [44] | Executive function; language; verbal memory; verbal learning; visuospatial learning; attention/working memory | 13            | 13            | 6.00                 | Gel             | 100 mg/d              | 29.31 (3.3) Normal               | Normal 411 (125.8) pM | Normal 541.9 (310.2) pM | 0.23       | Serum                | Direct RIA    | NR/NR                |
| Young et al.      | Executive function; language; verbal memory; verbal learning; visuospatial learning; attention/working memory | 15            | 15            | 6.00                 | Gel             | 75 mg/d               | 67.40 (5.5) Normal               | Normal 241 (85.5) pM | Normal 347.6 (155.2) pM | 1.06       | Serum                | Direct RIA    | NR/NR                |
| Borst et al. [58] | Executive function; visuospatial memory; visuospatial learning; attention/working memory | 30            | 19            | 32.00                | Injection       | 125 mg/wk             | 70.00 (8.9) Normal               | Low-normal 245.0 (73.0) ng/dL | Normal 474.0 (193.5) ng/dL | 1.46       | Serum                | Direct CIA    | NR/NR                |
| Cherrier et al.   | Executive function; language; verbal memory; visuospatial memory; verbal learning; visuospatial learning; attention/working memory | 22            | 19            | 24.00                | Derma gel       | 50–100 mg/d           | 70.50 (60–88) Impaired (MCI)     | Low-normal 308.2 (82.1) ng/dL | Normal 600.7 (19.7) ng/dL | 1.91       | Serum                | LC/MS/ MS     | Random/ not fasting |
| Huang et al. [60] | Executive function; language; verbal memory; visuospatial memory; verbal learning; visuospatial learning | 308           | 240           | 156.00               | Gel (1%) T gel/d | 67.55 (5.10) Normal | Low-normal 305.5 (63.4) ng/dL | Normal 567.7 (385.1) ng/dL | 1.40       | Serum                | Direct IA     | Morning/ fasting    |
| Melehan et al.    | Executive function; reaction time                                                | 67            | 54            | 18.00                | Injection       | 1000 mg/6 wk          | 49.00 (1.6) Normal              | Normal 392.7 (61.4) ng/dL | Normal 539.04 (15.16) ng/dL | 1.40       | Serum                | LC/MS/ MS     | Morning/ NR         |

(Continued)
| Study | Cognitive Outcome | Pre Post Duration | Type | Doses | Mean Age (Range/SD) | Gonadal Status Pre | T | Effect | T | Medium Assay Time |
|-------|-------------------|-------------------|------|-------|---------------------|-------------------|---|--------|---|------------------|
| Wahjoepramono et al. [62] | Cognitive status; verbal memory; verbal learning | 50 44 24.00 Cream (5%) | 50 mg/d | 61.05 (7.7) Normal Normal 474.4 (126.8) ng/dL | 1.40 Serum LC-MS | NR/NR |
| Resnick et al. [63] | Cognitive status; executive function; verbal memory; verbal learning; visuospatial learning; reaction time | 493 438 52.00 Gel (1%) | 5.00 g | 72.20 (6.0) Impaired (AAMI) Low–normal 234.4 (65.2) ng/dL | 1.80 Serum LC-MS | NR/NR |

Abbreviations: AAMI, age-associated memory impairment; AD, Alzheimer’s disease patients; CIA, chemiluminescent immunoassay; IA, immunoassay; LC, liquid chromatography; MCI, mild cognitively impaired patients; MS/MS, tandem mass spectrometry; NR, not reported; Post, post-treatment assessment; Pre, pretreatment assessment; RIA, radioimmunoassay; TS, testosterone.

a Number of participants randomized to the study.
b Number of participants completing the study.
c Duration of treatment is presented in weeks.
d Type refers to the type of treatment used to administer TS.
e Total sample mean age and associated range or SD depending on the available data.
f The treatment groups’ gonadal status before and after treatment was categorized depending on mean total testosterone (TT) levels as follows: (i) low, TT < 231 ng/dL; (ii) low–normal, TT between 232 and 320 ng/dL; (iii) normal, TT between 321 and 865 ng/dL; and (iv) supraphysiological (high), TT > 865 ng/dL. When only mean free testosterone (FT) levels were available, treatment groups’ gonadal status were categorized as the following: (i) low, FT < 174 pM; (ii) low–normal, FT between 175 and 220 pM; and (iii) normal, FT between 221 and 846 pM. In one case [49], only mean bioavailable testosterone (BT) levels were provided, and these were categorized as normal (BT between 72 and 235 ng/dL).
g The effect of TS on testosterone levels was calculated as an effect size (Hedges $g$) for the increase in serum testosterone in the active vs control group from pre- to post-treatment.
h Positive values indicate an effect in the expected direction, i.e., largest increase in the active groups’ testosterone levels compared with the control group.
i Lu et al. [43] presented separate data on Alzheimer’s disease patients and healthy men, and the reference is thus represented with two different studies in the table.
j Young et al. [44] presented separate data on young men and older men, and the reference is thus represented with two different studies in the table.
k Treatment doses were adjusted to keep testosterone concentrations within specified ranges.

Positive values indicate an effect in the expected direction, i.e., largest increase in the active groups’ testosterone levels compared with the control group.
whereas six studies included men who had testosterone levels in the low–normal range (mean total testosterone between 232 and 320 ng/dL).

The combined effect of TS on the primary outcome of overall cognitive functioning was small and failed to reach statistical significance (Hedges $g = 0.09$; CI 95%: $-0.02$ to 0.19, $K = 23$; see Table 2 and Fig. 2). The pooled effect sizes for all individual cognitive domains were small and statistically nonsignificant ($g = -0.04$ to 0.19, $P = 0.061$ to 0.989). When studies were grouped according to the proposed categorical moderators, small and statistically significant pooled effects were found for the following: (i) studies assessing men with mean testosterone levels within the normal range at baseline ($g = 0.17$; CI 95%: 0.00 to 0.33, $K = 17$), (ii) studies assessing younger men (mean age <68 years; $g = 0.20$; CI 95%: 0.02 to 0.36, $K = 12$), (iii) studies administering testosterone with injection ($g = 0.25$; CI 95%: 0.04 to 0.57, $K = 11$), (iv) studies assessing testosterone at random times ($g = 0.32$; CI 95%: 0.02 to 0.61, $K = 6$), and (v) nonindustry-sponsored studies ($g = 0.29$; CI 95%: 0.06 to 0.52, $K = 10$). Heterogeneity generally appeared to be low to moderate ($I^2 = 0.0$ to 59.3).

The risk of bias assessment was challenged by insufficient reporting, particularly of details related to methods of randomization and allocation concealment (see Table 3). Only five studies were preregistered online, limiting the assessment of selective reporting. Industry sponsorship was reported in 10 studies. The authors of 12 papers were contacted by E-mail to clarify study characteristics, of which four responded. Although the result for the primary outcome of overall cognitive functioning did not reach statistical significance, a funnel plot suggested possible publication bias in the direction of positive outcomes (Egger test, $P = 0.053$). The effects of four “missing” studies were imputed, resulting in an adjusted effect approaching zero ($g = 0.04$; Fig. 3). Although all fail-safe ns for the five statistically significant results failed to meet the criterion (Table 2), there were no indications of publication bias.

When explored with meta-regression in unadjusted models, the effect of TS on testosterone emerged as a statistically significant moderator of the overall pooled effect, with studies with larger effects of TS on testosterone showing stronger effects on cognitive functioning ($B = 0.09$). None of the other suggested moderators of the overall pooled effect reached statistical significance (Table 4). The results for three moderators showed a statistical trend ($P < 0.10$). This included TS type, with studies using gel/cream and studies using pellets, both showing weaker effects than studies using injection ($B = -0.27$ and $-0.36$). A trend toward larger effects was found for studies assessing testosterone at random times compared with those assessing testosterone in the morning ($B = 0.27$), and a trend for weaker effects was found for industry-sponsored studies compared with studies not sponsored by the industry ($B = -0.23$). When the effect of TS on testosterone was explored as a moderator of the overall pooled effects in studies with younger and older men, respectively, a trend toward studies with larger effects of TS on testosterone, showing stronger effects on cognitive functioning ($B = 0.14$, $K = 11$), was found in the subgroup of studies assessing older men. When intercorrelated moderators were explored in adjusted models, the effect of TS on testosterone levels was no longer a statistically significant moderator of the overall pooled effect after adjusting for TS type and time of day of testosterone measurement, respectively ($B = 0.08$ and 0.07; see Table 4).

When imputing 0 as the effect size for five neuropsychological outcomes not reported in two included studies, the effects were reduced to statistical nonsignificance in four out of the five study subgroups with statistically significant effects, i.e., (i) studies assessing men with normal testosterone levels, (ii) studies assessing younger men, (iii) studies using injection, and (iv) studies measuring testosterone at random times during the day. When winsorizing the effects of two outlier studies, the results were generally similar to those obtained with nonwinsorized data. The exception was in the study subgroup investigating men with normal testosterone levels, where the effect failed to reach significance when outliers were winsorized (see Table 5).

3. Discussion

Our meta-analysis of 23 RCTs of the effect of TS on cognitive functioning in men revealed a negligible (Hedges $g = 0.09$) and statistically nonsignificant effect on the primary outcome of
Table 2. Pooled Effect Sizes Across Outcomes

| Outcome                             | Sample Size | Heterogeneity \(^a\) | Effect Size \(^b\) | Fail-Safe n \(^c\) | Criterion \(^d\) |
|-------------------------------------|-------------|-----------------------|--------------------|---------------------|-----------------|
|                                     | K  n        | Q  df  P  I\(^2\)  g  95% CI  P   |                    |                     |                 |
| Overall combined effect             | 23 1638     | 20.34 22 0.562 0.00 0.09 -0.02 to 0.19 0.108 | —  —               |                     |                 |
| Cognitive domain                    |             |                       |                    |                     |                 |
| Attention/working memory            | 11 488      | 7.028 10 0.723 0.00 0.16 -0.07 to 0.33 0.06 | —  —               |                     |                 |
| Executive function                  | 6 657       | 1.24 5 0.624 0.00 -0.04 -0.19 to 0.11 0.634 | —  —               |                     |                 |
| Language                            | 7 423       | 9.64 6 0.141 37.77 0.07 -0.11 to 0.24 0.442 | —  —               |                     |                 |
| Verbal learning                     | 10 1201     | 5.51 9 0.788 0.00 0.03 -0.62 to 0.11 0.572 | —  —               |                     |                 |
| Verbal memory                       | 16 1378     | 23.11 15 0.082 35.10 0.08 -0.06 to 0.22 0.256 | —  —               |                     |                 |
| Visuospatial function               | 10 563      | 22.12 9 0.009 59.31 0.15 -0.14 to 0.43 0.311 | —  —               |                     |                 |
| Visuospatial function               | 10 878      | 3.99 9 0.912 0.00 0.00 -0.11 to 0.12 0.989 | —  —               |                     |                 |
| Visuospatial learning               | 7 902       | 10.82 6 0.094 44.56 0.19 -0.12 to 0.49 0.234 | —  —               |                     |                 |
| Visuospatial memory                 | 7 454       | 6.81 6 0.339 11.92 0.05 -0.11 to 0.22 0.525 | —  —               |                     |                 |
| Visuospatial skills (combined) \(^f\) | 16 1322     | 12.25 15 0.660 0.00 0.04 -0.06 to 0.15 0.420 | —  —               |                     |                 |
| Participants' cognitive status      |             |                       |                    |                     |                 |
| Cognitively normal                  | 18 1072     | 15.21 17 0.580 0.00 0.14 -0.01 to 0.28 0.06 | —  —               |                     |                 |
| Cognitively impaired                | 5 566       | 4.09 4 0.394 2.26 0.03 -0.15 to 0.21 0.739 | —  —               |                     |                 |
| Participants gonadal status         |             |                       |                    |                     |                 |
| Normal                              | 17 571      | 18.06 16 0.320 11.42 0.17 0.00 to 0.33 0.048 | 7 95                |                     |                 |
| Low/normal                          | 6 1067      | 0.69 5 0.983 0.00 0.02 -0.13 to 0.17 0.785 | —  —               |                     |                 |
| Age dichotomized                    |             |                       |                    |                     |                 |
| Young age (<68 y)                   | 12 591      | 10.50 11 0.486 0.00 0.20 0.02 to 0.36 0.032 | 7 70                |                     |                 |
| Old age (≥68 y)                     | 11 1048     | 7.65 10 0.663 0.00 0.03 -0.10 to 0.16 0.676 | —  —               |                     |                 |
| Administration type                 |             |                       |                    |                     |                 |
| Gel/cream                           | 8 914       | 2.40 7 0.934 0.00 0.02 -0.13 to 0.16 0.839 | —  —               |                     |                 |
| Pellets                             | 2 281       | 1.26 1 0.263 20.34 -0.11 -0.50 to 0.28 0.593 | —  —               |                     |                 |
| Injection                           | 11 325      | 11.18 10 0.343 10.59 0.25 0.04 to 0.57 0.021 | 10 65               |                     |                 |
| Time of measure                     |             |                       |                    |                     |                 |
| Morning                             | 9 1277      | 9.86 8 0.275 18.86 0.07 -0.09 to 0.23 0.378 | —  —               |                     |                 |
| Not stated                          | 8 181       | 3.00 7 0.941 0.00 0.07 -0.20 to 0.34 0.608 | —  —               |                     |                 |
| Random                              | 6 180       | 5.37 5 0.372 6.92 0.32 0.02 to 0.61 0.037 | 3 40                |                     |                 |
| T measurement assay                 |             |                       |                    |                     |                 |
| LC-MS/MS                            | 4 611       | 2.18 3 0.536 0.00 0.03 -0.12 to 0.18 0.691 | —  —               |                     |                 |
| Direct CIA/RIA                      | 19 1027     | 17.15 18 0.513 0.00 0.14 -0.01 to 0.29 0.064 | —  —               |                     |                 |

(Continued)
Table 2. Pooled Effect Sizes Across Outcomes (Continued)

| Outcome                        | Sample Size | Heterogeneity | Effect Size | Fail-Safe n | Criterion |
|-------------------------------|-------------|---------------|-------------|-------------|-----------|
|                               | K  | n  | Q  | df | P  | I² | g  | 95% CI | P  |           |           |
| Publication year               |    |    |    |    |    |    |    |    |    |           |           |
| Early studies                 | 11 | 360| 15.63 | 10 | 0.111 | 36.01 | 0.24 | −0.02 to 0.49 | 0.069 | —          | —          |
| Late studies                  | 12 | 1278| 3.02 | 11 | 0.990 | 0.00 | 0.04 | −0.09 to 0.17 | 0.524 | —          | —          |
| Industry sponsoring            |    |    |    |    |    |    |    |    |    |           |           |
| Sponsored                     | 12 | 1254| 3.91 | 11 | 0.972 | 0.00 | 0.05 | −0.08 to 0.18 | 0.470 | —          | —          |
| Not sponsored                 | 10 | 308 | 10.37 | 9  | 0.322 | 13.18 | 0.29 | 0.06 to 0.52 | 0.014 | 12         | 60         |

Abbreviations: df, degrees of freedom; K, number of studies in the analysis; n, number of subjects in the analysis.

a Q statistic, P < 0.1 taken to suggest heterogeneity (bold) [33]; I² statistic, 0% (no heterogeneity), 25% (low heterogeneity), 50% (moderate heterogeneity), 75% (high heterogeneity) [31].

b Effect size = Hedges g. A positive value indicates an effect size in the hypothesized direction, i.e., improvement in the active group’s cognitive functions compared with the control group. Conventions: small (0.2), medium (0.5), and large (0.8) [28]. Statistically significant P (<0.05) is in bold. Statistically trending P (>0.05 <0.10) is in italics.

c In case of statistically significant effects, the robustness of findings was examined by calculation of the fail-safe n (number of nonsignificant studies that would bring P to >0.05) [36].

d Fail-safe n exceeding the criterion (5K + 10) indicates a robust result [36].

e Cognitive domains using visuospatial skills (visuospatial function, visuospatial learning, and visuospatial memory) were pooled.
overall cognitive functioning. When further taking possible publication bias into consideration, the pooled effect approached zero. Five (17%) of the 29 secondary analyses of various study subsets reached statistical significance, including studies assessing men with normal testosterone levels, studies assessing younger men (age < 68 years), studies administering testosterone with injection, nonindustry-sponsored studies, and studies assessing testosterone at random times. The latter subgroup is of interest, as this suboptimal assessment of testosterone [64] may affect the estimate of TS effects on testosterone levels, which emerged as the only statistically significant moderator of the overall pooled effect when explored with meta-regression. The statistically significant effects all failed to meet the fail-safe number criterion, indicating less than robust results. The current study highlights several questions that remain unanswered and should be investigated further.

First, it may have seemed optimistic to expect an effect of TS on cognitive functions in men with sufficient physiological testosterone at the outset. Rather than the examination of the effects of TS in men with physiological levels within normal ranges, it would arguably be of most interest to clinicians to know whether testosterone replacement has an effect in men with clinical hypogonadism. Unfortunately, we were unable to examine this, as none of the included studies treated samples of men with clinically low testosterone levels. However, six
studies examined men with testosterone in the low end of the normal range. Surprisingly, the pooled effect of these studies were negligible and statistically nonsignificant ($g = 0.02$), opposed to the small and statistically significant effect ($g = 0.17$; $P = 0.048$) found for the 17 studies of men with mean testosterone levels within the normal range. However, there are several reasons this could be a chance finding. First, the results were less robust, i.e., did not reach the fail-safe number criterion. Second, the effect no longer reached statistical significance.

Table 3. Risk of Bias of Included Studies

| Study Reference          | Random Sequence Generation | Allocation Concealment | Blinding of Participants and Personal | Incomplete Outcome Data | Selective Reporting | Pharmaceutical Industry Sponsor |
|--------------------------|-----------------------------|------------------------|--------------------------------------|------------------------|---------------------|-------------------------------|
| Janowsky et al. (45)     | ★                           | ●                      | ★                                    | ★                      | ●                   | ●                             |
| Janowsky et al. (46)     | ★                           | ●                      | ★                                    | ★                      | ●                   | ●                             |
| Cherrier et al. (47)     | ●                           | ●                      | ●                                    | ●                      | ●                   | ●                             |
| O’Connor et al. (48)     | ●                           | ●                      | ●                                    | ●                      | ●                   | ●                             |
| Kenny et al. (49)        | ●                           | ●                      | ●                                    | ●                      | ●                   | ●                             |
| Kenny et al. (50)        | ●                           | ●                      | ●                                    | ●                      | ●                   | ●                             |
| Cherrier et al. (51)     | ●                           | ●                      | ●                                    | ●                      | ●                   | ●                             |
| Cherrier et al. (52)     | ●                           | ●                      | ●                                    | ●                      | ●                   | ●                             |
| Haren et al. (53)        | ●                           | ●                      | ●                                    | ●                      | ●                   | ●                             |
| Lu et al. (43)           | ●                           | ●                      | ●                                    | ●                      | ●                   | ●                             |
| Cherrier et al. (54)     | ●                           | ●                      | ●                                    | ●                      | ●                   | ●                             |
| Maki et al. (55)         | ●                           | ●                      | ●                                    | ●                      | ●                   | ●                             |
| Vaughan et al. (56)      | ●                           | ●                      | ●                                    | ●                      | ●                   | ●                             |
| Emmelot-Vonk et al. (57) | ●                           | ●                      | ●                                    | ●                      | ●                   | ●                             |
| Young et al. (44)        | ●                           | ●                      | ●                                    | ●                      | ●                   | ●                             |
| Borst et al. (58)        | ●                           | ●                      | ●                                    | ●                      | ●                   | ●                             |
| Cherrier et al. (59)     | ●                           | ●                      | ●                                    | ●                      | ●                   | ●                             |
| Huang et al. (60)        | ●                           | ●                      | ●                                    | ●                      | ●                   | ●                             |
| Melehan et al. (61)      | ●                           | ●                      | ●                                    | ●                      | ●                   | ●                             |
| Wahjoepramono et al. (62)| ●                           | ●                      | ●                                    | ●                      | ●                   | ●                             |
| Resnick et al. (63)      | ●                           | ●                      | ●                                    | ●                      | ●                   | ●                             |

Studies were rated as having ★ low risk, ● high risk, or ○ unclear risk of bias using the Cochrane Collaboration tool for assessing risk of bias [29].
significance when we imputed zero as the effect size for five neuropsychological outcomes not reported in two studies. Finally, the result failed to reach statistical significance when winsorizing the outlier effect sizes of two studies. Furthermore, we have no clear explanation for our findings of statistically significant effects in the two subsets of studies investigating younger men (age $\leq 68$ years) and studies assessing testosterone at random times during the day. Regarding men of younger age, we would expect these to benefit less from TS than men of older age who are more likely to have low levels of physiological testosterone and reduced cognitive reserve as a result of aging. Likewise, whereas the assessment time may affect the estimate of the effect of TS on testosterone levels, we see no apparent reason for a direct moderating effect of testosterone assessment time on the effect of TS on overall cognitive functioning. Given this lack of theoretical support for the findings, in addition to the lack of robustness of the results, we are inclined to believe these are chance findings.

Second, it remains to be explored in detail whether TS may actually be beneficial to cognitive functioning in the context of neurodegenerative disease. Whereas we found a smaller effect in subgroup analyses of studies with cognitively impaired men ($g = 0.03$) compared with studies of cognitively normal men ($g = 0.14$), only five studies assessed cognitively impaired men ($n = 566$) with the largest of these studies [63], including men with “age-associated memory impairment” (AAMI) ($n = 493$). As AAMI excludes men with neuropsychological test scores 2 SDs below the scores of age-matched men on tests of paragraph recall or visual memory [63], these participants were unlikely to suffer from neurodegenerative diseases. Thus, rather than the representation of the effect of TS on cognitive functioning in men with neurodegenerative diseases, the subgroup analysis of cognitively

![Funnel Plot of Precision by Hedges g](image)

**Figure 3.** Funnel plot of the overall pooled effect with missing studies imputed. Funnel plot of precision [1/SE (Std Err)] by effect sizes (Hedges $g$) with imputed studies. Each open circle represents precision as a measure of the sample size on the y-axis as a function of the effect size of each independent study on the x-axis. Closed circles represent imputed studies. The open diamond in the bottom of the plot indicates the summary effect size of the analysis. The closed diamond indicates the summary effect size with imputed studies included. A 95% CI is indicated by the lines. Within these lines, 95% of the circles are expected to be located. The overall pooled effect size is indicated by a vertical line in the middle of the plot.
impaired men in the present meta-analysis represents an estimate of the effect of TS on men with various degrees of cognitive impairment ranging from AAMI and mild cognitive impairment to Alzheimer’s disease. Given the evidence that points to the protective effect of testosterone against Alzheimer’s neuropathology, it would be of interest to examine the effect of TS in a larger and more homogeneous group of patients with neurodegenerative diseases.

Third, whether TS will have a beneficial effect on cognitive functioning may also depend on how it is administered. The most recent trial [63] administered TS using gel and found no effect on cognitive functions. It has been speculated that an injectable type of TS that leads to spikes of testosterone, rather than a gel-based type that releases a steady dose, could have a transient effect on cognitive functioning [65]. This is supported by research indicating that transdermal TS is not always adequately absorbed [66], which is further supported by the present meta-analysis showing the largest, statistically significant effect in the subgroup of studies that used injection (\( g = 0.25 \)), in addition to the statistically trending meta-regression findings of weaker effects in studies using gel/cream and pellets compared with injection.

Table 4. Results From Meta-Regression Analyses

| Variable                               | Unadjusted Models* | Adjusted Modelsb |
|----------------------------------------|--------------------|------------------|
|                                        | K      | B*    | 95% CI     | P     | B*    | 95% CI     | P     | VIFd  |
| Treatment characteristics             |        |       |            |       |       |            |       |       |
| TS Type                               | 23     |       |            |       |       |            |       |       |
| Gel/cream (vs injection)              |        | -0.27 | -0.47 to 0.02 | 0.073 |       |            |       |       |
| Patches (vs injection)                |        | -0.01 | -0.41 to 0.38 | 0.949 |       |            |       |       |
| Pellets (vs injection)                |        | -0.36 | -0.76 to 0.04 | 0.080 |       |            |       |       |
| Treatment duration, wk                | 23     |       |            |       |       |            |       |       |
|                                        |        | -0.00 | -0.00 to 0.00 | 0.336 |       |            |       |       |
| TS effect (Hedges g)                  | 23     | 0.09  | 0.01 to 0.19 | 0.044 | 0.08  | -0.03 to 0.19 | 0.140 | 1.47  |
|                                        |        |       |            |       |       | 0.07      | -0.33 to 0.18 | 0.180 | 1.37  |
|                                        |        |       |            |       |       | Adj. TS type |       |       |
| TS effect, young age (<68 y)          |        | 12    |       | 0.08  | -0.04 to 0.19 | 0.183 |       |       |       |
| TS effect, old age (≥68 y)            |        | 11    |       | 0.14  | -0.02 to 0.30 | 0.077 |       |       |       |
| T measurement characteristics         |        | 23     |       |       |       |            |       |       |
| Time of measurement                   |        |       |            |       |       |            |       |       |
| Random times (vs morning)             |        | 23    |       | 0.27  | -0.05 to 0.58 | 0.095 |       |       |       |
| Not stated (vs morning)               |        | 0.02  | -0.27 to 0.32 | 0.877 |       |            |       |       |
| Measurement assay                     |        | 23     |       |       |       |            |       |       |
| Direct CIA/RIA (vs LC-MS/MS)          |        | 0.11  | -0.10 to 0.32 | 0.317 |       |            |       |       |
| Participant characteristics           |        |       |            |       |       |            |       |       |
| Mean age                               |        | 23     |       | 0.00  | -0.01 to 0.01 | 0.758 |       |       |       |
| Cognitive status                      |        | 23     |       |       |       |            |       |       |
| Impaired (vs normal)                  |        | -0.11 | -0.33 to 0.10 | 0.309 |       |            |       |       |
| Gonadal status                        |        | 23     |       |       |       |            |       |       |
| Low–normal (vs normal)                |        | -0.14 | -0.35 to 0.08 | 0.208 |       |            |       |       |
| Study characteristics                 |        |       |            |       |       |            |       |       |
| Early publication (vs late)           |        | 23     |       | -0.15 | -0.39 to 0.08 | 0.194 | -0.19 |       | -0.49 to 0.11 | 0.205 | 1.68  |
| Sponsored (vs not sponsored)         |        | 22    |       | -0.23 | -0.48 to 0.02 | 0.067 | -0.13 |       | -0.42 to 0.18 | 0.407 | 1.66  |

Statistically significant \( P (<0.05) \) is in bold. Statistically trending \( P (>0.05 <0.10) \) is in italic.

Abbreviations: Adj., adjusted; VIF, variance inflation factor.

*Variables were explored individually in unadjusted models.

\( ^{b} \)When theoretically sound intercorrelations between variables reached statistical significance \( (P < 0.05) \), they were explored together in adjusted models.

\( ^{c} \)The association between moderators and the magnitude of the effect is expressed in unstandardized regression coefficients (B).

\( ^{d} \)The variance inflation factor (VIF), a measure of multicollinearity, was calculated when variables were explored together in adjusted models. Conventions: VIF > 10 indicates a serious problem with bias [39, 40]. If VIF is substantially greater than one, then the regression may be biased [40].
Overall, found in nonindustry-sponsored trials (reported in industry-sponsored trials were of a smaller magnitude (g = 0.05) than the effects found in nonindustry-sponsored trials (g = 0.29); the difference approaching statistical significance (P = 0.075). Whereas we have no clear explanation, a post hoc analysis revealed that studies published in 2007 and later were more likely to be industry sponsored than the early studies published before 2007. As the effects found in early studies (g = 0.24) were larger than in later studies (g = 0.04), this could be a partial explanation for the unexpected finding.

Finally, the hypothesis that TS should benefit cognitive functioning may be overly simplistic. It has been suggested that the association between low testosterone and increased

| Outcome Analyses of Statistically Significant Effects With Original and Revised Pooled Effects |
|---------------------------------------------------------------|
| **Outcome** | **Sample Size** | **Heterogeneity** | **Effect Size** | **Fail-Safe n** | **Criterion** |
|-------------|----------------|-----------------|----------------|----------------|--------------|
| Overall    | Original 23 1638 | 20.34 22 0.562 0.00 | 0.09 -0.02 to 0.19 0.108 | — | — |
| cognitive | Imputed 23 1638 | 14.71 22 0.874 0.00 | 0.07 -0.04 to 0.18 0.189 | — | — |
| functioning | Winsorized 23 1638 | 18.24 22 0.692 0.00 | 0.07 -0.03 to 0.16 0.169 | — | — |
| Normal     | Original 17 571 | 18.06 16 0.320 11.42 | 0.17 0.00 to 0.33 0.048 | 7 95 | — |
| gonadal   | Imputed 17 571 | 16.17 16 0.441 10.79 | 0.14 -0.01 to 0.30 0.065 | — | — |
| status     | Winsorized 17 571 | 15.33 16 0.501 0.00 | 0.16 -0.00 to 0.31 0.054 | — | — |
| Young age | Original 12 591 | 10.50 11 0.486 0.00 | 0.20 0.02 to 0.36 0.032 | 7 70 | — |
| Using      | Imputed 12 591 | 7.71 11 0.739 0.00 | 0.17 -0.01 to 0.35 0.062 | — | — |
| injection  | Winsorized 12 591 | 9.71 11 0.557 0.00 | 0.18 0.02 to 0.35 0.033 | 6 70 | — |
| Random     | Original 11 325 | 11.18 10 0.343 10.59 | 0.25 0.04 to 0.57 0.021 | 11 70 | — |
| times      | Imputed 11 325 | 6.97 10 0.728 0.00 | 0.19 -0.01 to 0.38 0.068 | — | — |
| Not sponsored | Winsorized 11 325 | 11.19 10 0.342 10.69 | 0.24 0.04 to 0.45 0.021 | 11 70 | — |

Sensitivity analyses of the primary outcome, i.e., the effect of TS on overall cognitive functioning, defined as the individual cognitive domain scores aggregated into one combined estimate, and of five study subsets showing statistically significant improvements: (i) studies of men with mean testosterone levels within normal range (total testosterone between 321 and 865 ng/dL), i.e., normal gonadal status; (ii) studies of younger men (age <68 y); (iii) studies administering testosterone with injection; (iv) studies assessing testosterone at random times; and (v) trials not sponsored by the industry.

A positive value indicates an effect size in the hypothesized direction, i.e., improvement in the active group's cognitive functions compared with the control group. Conventions: small (0.2); medium (0.5); large (0.8) [28]. Statistically significant P (<0.05) is highlighted in bold. Statistically trending P (>0.05 <0.10) is highlighted in italic.

In case of statistically significant effects, the robustness of findings was examined by calculation of the fail-safe n (number of nonsignificant studies that would bring P to >0.05) [36].

Fail-safe n exceeding the criterion (5K + 10) indicates a robust result [36].

Imputed = imputed effect size of zero in two cases [47, 52], when included publications stated they had obtained nonsignificant results on one or more neuropsychological tests without presenting the data.

A range of 2 SDs below and above the global effect size was used to truncate outliers. In two study subsets, i.e., studies assessing testosterone at random times and studies not sponsored by the industry, no studies were below or above this range; thus, no sensitivity analyses with truncated outliers were conducted.

(B = -0.27 and -0.36). However, the difference between routes of administration may be a result of the effect of treatment on circulating testosterone and thus, the effect of testosterone on different organs, and it may also be a matter of differing doses given. In other words, this issue may only be resolved by head-to-head studies examining injection vs transdermal administration aiming at dose equivalency.

Fourth, yet another unanswered question relates to the concept that whereas industry sponsorship of pharmacotherapy trials is associated with more favorable outcomes [30], the effects reported in industry-sponsored trials were of a smaller magnitude (g = 0.05) than the effects found in nonindustry-sponsored trials (g = 0.29); the difference approaching statistical significance (P = 0.075). Whereas we have no clear explanation, a post hoc analysis revealed that studies published in 2007 and later were more likely to be industry sponsored than the early studies published before 2007. As the effects found in early studies (g = 0.24) were larger than in later studies (g = 0.04), this could be a partial explanation for the unexpected finding.

Finally, the hypothesis that TS should benefit cognitive functioning may be overly simplistic. It has been suggested that the association between low testosterone and increased
risk for Alzheimer’s may not stem from testosterone depletion per se but rather from an increase in serum gonadotropins as a result of loss of negative regulation of testosterone on the hypothalamus and pituitary [67]. Consequently, rather than the focus solely on TS, future studies may need to focus on attempts to balance the dynamics of the hypothalamic-pituitary axis and thereby all sex hormones [65]. It also seems relevant to examine the role of estradiol for cognitive functioning in men, as estradiol primarily stems from the aromatization of testosterone and plays an important role in bone metabolism, body composition, and sexual function [68, 69].

A. Strengths and Limitations

We conducted this review based on an a priori-defined protocol and used a rigorous methodological approach. Among additional strengths are the comprehensive search strategy; the relatively homogenous studies available, as indicated by heterogeneity statistics; and our detailed examination of the possible role of between-study methodological differences. Some limitations should also be noted. First, our search was limited to English-language publications and did not include the “grey literature.” English language as an inclusion criterion could possibly introduce a risk of overlooking important results reported in other languages. However, as we would be unable to include all languages as a result of restricted language competencies in the group of authors, this could in itself introduce additional bias. Furthermore, there is evidence to suggest that English-language restriction does not introduce systematic bias. A systematic review of reviews examining a total of 361 meta-analyses of studies thus found no evidence of a systematic bias from the use of language restrictions in systematic review-based meta-analyses in conventional medicine [70]. It is also worth noting that in the present review, none of the 220 non-English records identified in the databases that were excluded during the initial abstract screening met the remaining inclusion criteria. Concerning the grey literature, the inclusion of data from unpublished studies can itself introduce bias, as the studies that can be identified may be an unrepresentative sample of all unpublished studies [29]. Second, we categorized patients in included studies as having “low,” “low–normal,” “normal,” or “supraphysiological” testosterone, respectively, depending on reported mean total testosterone levels. This approach has several drawbacks, given that reported ranges for testosterone concentrations vary among laboratories and assays and that testosterone levels should not be evaluated in isolation without taking important confounding factors, such as sexual hormone-binding globulin levels, into account [64]. However, the included papers did not present sufficient information to enable us to evaluate variations in reference ranges, and whereas some papers presented sexual hormone-binding globulin levels, this was as mean statistics only, thus not allowing for individually based evaluations. Despite these challenges, we attempted to categorize studies depending on testosterone levels to give an indication of the hypogonadal status of the participants. For the sake of transparency, we also reported the mean testosterone levels and associated SDs together with the hypogonadal categories in Table 1. Additional limitations relating to the available literature itself included the small number of available studies included in some of the subgroup analyses, making it difficult to draw robust conclusions regarding subgroup differences. Our ability to evaluate the strength of the evidence was generally limited, as many reports, in particular, the early studies, had not been preregistered online and failed to provide sufficient information concerning randomization and allocation concealment.

B. Strengths and Weaknesses in Relation to Other Studies

Six previous narrative reviews of the relationship between testosterone and cognitive functioning in men are relevant for comparison [15–20]. Of these, only one [16] included only RCTs and thus, appears to be the most comparable with the present review, which was also restricted to RCTs. That review [16] evaluated 156 RCTs assessing the effect of TS on various outcomes, including cognition (K = 23). In accordance with our findings, the authors
concluded that prescription of TS for improving cognitive functioning is without support from the available evidence. However, the review may be limited in several ways. First, in addition to studies assessing cognitive functioning by means of objective neuropsychological testing, the review also included studies assessing cognitive functioning by means of self-reports only. This may be problematic as self-reported cognitive functioning is more likely to reflect emotional distress rather than objective cognitive impairment. Often, self-reported measures of cognition tend not to be highly correlated with objective neuropsychological outcomes [71–73]. Second, as the remaining five previous narrative reviews [15, 17–20], that systematic review [16] was limited to simple vote counting of statistically significant results when evaluating the effect of TS on cognitive functioning in men. In contrast, in the present review, we have evaluated the effect of TS on cognitive functioning by means of meta-analysis, which more accurately estimates the overall effect of TS on cognitive functioning. The present systematic review with meta-analysis addresses the efficacy of TS on cognitive functioning in men, thus, addressing a gap in the existing knowledge within the field.

C. Meaning of the Study—Explanations and Implications

Taken together, the available RCTs do not support a beneficial effect of TS on cognitive functioning in men with testosterone levels within normal ranges. There is, thus, no evidence for prescribing TS for improving cognitive functioning in men with no clinical signs of hypogonadism, in particular, when considering the potential adverse effects and the inadequately understood risk for cardiovascular events associated with TS [64, 74]. Of five statistically significant subgroup analyses, four were unexplainable and appear to be chance findings given the lacking robustness of the results. Future studies could investigate whether TS may have positive effects on cognitive functioning in cases where testosterone replacement is indicated to induce and maintain secondary sex characteristics and correct symptoms of hypogonadism and where treatment is continued for a sufficient length of time for it to have a detectable effect on cognitive functions.

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