EXPERT OPINION

Cardiovascular risk associated with testosterone-boosting medications: a systematic review and meta-analysis

Giovanni Corona, Elisa Maseroli, Giulia Rastrelli, Andrea M Isidori, Alessandra Sforza, Edoardo Mannucci & Mario Maggi†

†*University of Florence, Department of Experimental, Clinical and Biomedical Sciences, Sexual Medicine and Andrology Unit, Florence, Italy*

Introduction: Recent reports have significantly halted the enthusiasm regarding androgen-boosting; suggesting that testosterone supplementation (TS) increases cardiovascular (CV) events.

Areas covered: In order to overcome some of the limitations of the current evidence, the authors performed an updated systematic review and meta-analysis of all placebo-controlled randomized clinical trials (RCTs) on the effect of TS on CV-related problems. Out of 2747 retrieved articles, 75 were analyzed, including 3016 and 2448 patients in TS and placebo groups, respectively, and a mean duration of 34 weeks. Our analyses, performed on the largest number of studies collected so far, indicate that TS is not related to any increase in CV risk, even when composite or single adverse events were considered. In RCTs performed in subjects with metabolic derangements a protective effect of TS on CV risk was observed.

Expert opinion: The present systematic review and meta-analysis does not support a causal role between TS and adverse CV events. Our results are in agreement with a large body of literature from the last 20 years supporting TS of hypogonadal men as a valuable strategy in improving a patient's metabolic profile, reducing body fat and increasing lean muscle mass, which would ultimately reduce the risk of heart disease.

Keywords: cardiovascular risk, mortality, randomized clinical trial, testosterone

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1. Introduction

Although testosterone (T) levels naturally decline with aging – and therefore more and more elderly men with T deficiency are accumulating worldwide because of prolonged life expectancy – it is still questionable whether the increased T prescription rate reflects the real clinical scenario, that is, increased global prevalence of hypogonadism [1-5]. More likely, in westernized societies with a youth-oriented culture, a widespread awareness of the side effects of T deficiency results in a perception of T as a hormonal, easy-to-take, fountain of youth and therefore as a genuine source of vigor and virility. The latter point, which is particularly appealing, is mainly supported by the clinical observation that hypogonadal men often complain of sexual symptoms and a lack of vigor. In fact, in a recent European survey (European Male Aging Study, [5]), hypoactive sexual desire, loss of spontaneous and sex-related erections were associated, in a syndromic way, with low T (see below). Lack of vigor (i.e., inability to engage in vigorous activity, inability to walk for long distances and an inability to bend) and psychological disturbances (i.e., loss of energy, sadness and fatigue) were also associated with T deficiency,

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Article highlights.

- Recent reports suggested that testosterone supplementation (TS) might increase cardiovascular (CV) risk.
- The authors performed here a new systematic review and meta-analysis on all published and unpublished randomized controlled trials reporting the effects of TS on different CV outcomes.
- Our analysis indicates that TS is not related to any increase in CV risk even when composite or single adverse events were considered.
- When studies performed in subjects with metabolic derangements were analyzed separately a protective effect of TS on CV risk was observed.
- Retrieved trials included 3016 TS treated and 2448 placebo treated men for a mean duration of 34 weeks.
- Present data do not support a causal role between TS and CV events.
- TS of hypogonadal men could be a valuable strategy to improve metabolic profile, reducing body fat and increasing lean muscle mass that would ultimately reduce the risk of heart disease.

This box summarizes key points contained in the article.

but the relationships were weaker than for the sexual complaints. Several intervention studies [6-9] corroborate the notion that T substitution is able to ameliorate sexual dysfunction, including hypoactive sexual desire disorder and erectile dysfunction, at least in hypogonadal men [8,9]. However, the positive effects of boosting T in subjects without T deficiency are not evidence-based, at least when analyzed in meta-analytic surveys [8,9]. Nonetheless, T preparations are often inadequately prescribed – frequently without previously checking endogenous levels of the hormone – in an empirical attempt to improve sexual performance, energy and muscle mass, bone strength and mood [4]. In fact, although the hormone is FDA-approved for hypogonadism substitution only, T is widely marketed for treating symptoms compatible with 'low T syndrome', including fatigue, low libido and loss of energy, common conditions often faced by senior subjects [10], in the absence of a proper biochemical confirmation of hypogonadism. This attitude (in particular in the US), together with aggressive promotional campaigns and advertising, favor self-medication with the hope of improving sex drive and diminished energy, with the final effect that T sales in the last decade have soared.

2. Androgen boosting increases cardiovascular risk

Concerns about cardiovascular (CV) safety of T administration have always existed, because T is acknowledged to reduce HDL-cholesterol and to raise hematocrit [1-4]. In addition, recent reports in the scientific and lay press have further blunted the enthusiasm on androgen boosting, suggesting that testosterone supplementation (TS) increases CV risk [11-13]. The Endocrine Society released a statement indicating that "until evidence from large randomized trials becomes available, patients should be made aware of the potential risk of CV events in middle-aged and older men who are taking, or considering, TS for age-related declines in T levels and symptoms. Physicians and patients should have a conversation about the risks and benefits of using T, especially in patients who have pre-existing heart disease. The Endocrine Society recommends that physicians prescribe T in accordance with the Society's clinical practice guidelines on TS in men with hypogonadism". However, 'patients with hypogonadism who have been on stable TS should not stop their medication without consulting their healthcare provider' [14].

The numerous press claims, highlighting cardiac problems as a potential side effect, are essentially based on three articles, published in prestigious international journals in the last 4 years [11-13]. However, all these studies have important flaws, despite the quality and the impact of the journals publishing them. Nonetheless, the FDA on the basis of these studies [11-13] urged 'healthcare professionals and patients to report side effects involving prescription T products to the FDA MedWatch program'.

3. T and CVD: the evidence

The complex, and often multidirectional, interconnections between T and CV health are essentially based on epidemiological, community-based population studies and intervention studies, the latter further divided into placebo-controlled randomized trials and observational or pharmaco-epidemiological studies. The aim of this systematic review is to summarize them and providing an expert opinion on their clinical relevance. To overcome marked between-study heterogeneity, systematic reviews and meta-analyses will be particularly taken into consideration. Results from a new, updated meta-analysis on T-related CV events are also provided to overcome conflicting views from the previous ones.

4. Epidemiological studies

High T level was historically regarded as a risk factor for CV diseases (CVD) by inducing the typical risky behaviors associated with maleness [2,3]. Supporting this view, castration prolonged lifespan in eunuchs [15] and in mentally ill, institutionalized men [16]. However, Nieschlag and colleagues [17] comparing the lifespan of castrated and intact males did not reveal any differences in total or CV mortality. In addition, more recent epidemiological evidence suggests that reduced, rather than increased T levels could be associated with higher CVD [18,19]. In cross-sectional studies, T levels are lower in individuals with CVD than in the rest of population [20].

The difference between patients with CVD and healthy subjects appears to be greater among those with diabetes or hypertension [20]. Conversely, longitudinal observational studies demonstrated an association between hypogonadism and overall- and CV-mortality but not with incident CV events [20-22]. In addition, it has also been suggested that the increased risk associated with low T could be limited to elderly subjects [22]. Overall, epidemiological data suggest either no effect or a protective role of T with respect to CVD, even though increased testis volume is a positive predictor of unfavorable CV profile and incident CV events [23]. The interpretation of epidemiological data is complex because of the effect of confounders. For example, the association of low T and CV mortality could be due to the fact that they are both associated with aging. However, due to a large number of uncontrolled confounders, observational studies cannot be used to infer causal relationships, which are more properly addressed by randomized clinical trials (RCTs). Unfortunately, no long-term, prospective, intervention RCTs on TS with CV events as the primary end point are available to date. Only results from short-term studies, or obtained from RCTs designed for other purposes, are available and are the focuses of several meta-analyses.

5. Intervention studies

5.1 Intervention studies with CV events as the primary end point

Only few RCTs are available having CV events as primary end points. The authors recently meta-analyzed the six RCTs currently available, enrolling a small number of patients with coronary heart disease (CHD; n = 258), with a short follow-up (mean = 23 weeks) [20]. In CHD patients, TS was positively associated with a significant increase in treadmill test duration and time to 1-mm ST segment depression [20]. Similar results were obtained considering studies on TS in subjects with heart failure (HF) [24]. Results are summarized in **Table 1**. As for CHD, the available studies in HF were of short duration and follow-up. However, all reported significant improvements in exercise capacity after 12 – 52 weeks of TS. Meta-analysis of HF studies indicated a significant increase in exercise capacity by almost 54 m using the 6-min walk test. The authors commented that the effect of T was impressive and greater than that seen with other cardiological therapies routinely recommended in patients with HF [24].

5.2 Intervention studies without CV events as a primary end point

In contrast to trials with CV events as the primary end point, some data from trials designed for other purposes have generated concerns about the CV safety of TS. Of the four available meta-analyses (**Table 2**) [25-28], three [25-27] did not highlight any effect (either protective or harmful), of TS on CV events, although in one of them it was reported that T-treated subjects were nearly four times as likely as those in the placebo arm to develop a hematocrit > 50% [27]. Similarly, it has been suggested that TS could reduce HDL-cholesterol [1]. In contrast, a more recent meta-analysis [28] suggested a possible increased risk associated with T treatment. These meta-analyses were performed several years apart, thus including different sets of available studies – up to 2004 [25,26], 2008 [27] or 2012 [28]. In addition, the meta-analyses differed in criteria of trial inclusion, particularly for age limits and study duration (**Table 2**). More importantly, outcomes of interest, which were retrieved and analyzed, were different from one meta-analysis to another (**Table 2**). Notably, the only meta-analysis reporting an increased CV risk associated with TS [28] used a very broad composite end point, which included among CV events all investigator-reported adverse events assigned to the CV system. This procedure includes, among CV events, cases of 'peripheral edema' and 'self-reported syncope', leading to an artificial increase of the overall number of events. In addition, in the meta-analysis of Xu *et al.*, [28], the Basaria *et al.* trial [11], with the highest overall weight, is clearly discordant from all the others. The Basaria *et al.* study [11], which was performed with supra-physiological dose of T in elderly men with limitations in motility, also use a very broad definition of CV events, which are not adjudicated. The trial was prematurely terminated for concern over CV safety; however, its results are difficult to generalize. In fact, as TS enhances vigor [11] its administration in frail elderly men could induce an increase in physical activity, possibly leading to cardiac problems.

6. A new meta-analysis on clinical trials report

In order to overcome some of the limitations of the analysis of Xu *et al.*, [28], the authors performed an updated systematic review and meta-analysis of RCTs on TS, using a more conventional definition of CV events similar to that used by regulatory authorities to verify the safety of newly registered drugs [29]. This meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist (Supplementary file 1) [30].

6.1 Search strategy

An extensive Medline, Embase and Cochrane search was performed including the following words ('testosterone'[MeSH Terms] OR 'testosterone'[All Fields]) AND (Clinical Trial [ptyp] AND 'humans'[MeSH Terms] AND English[lang] AND 'male'[MeSH Terms]). The search, which accrued data from 1 January 1969 up to 31 January 2014, was restricted to TS placebo-controlled RCTs on different outcomes, English-language articles and studies of human participants. In addition, completed but still unpublished RCTs evaluating the effects of TS on different outcomes were identified through a search at www.clinicaltrials.gov website including the term 'testosterone'. The identification of relevant studies was performed independently by two of

Table 1. Mean difference or standardized mean difference in several clinical parameters after testosterone supplementation as derived from meta-analysis of the available evidence.

	Exercise duration (mean difference seconds)	Time to 1 mm ST depression (mean difference; seconds)	Exercise capacity (standardized difference)	Peak in VO_2 (standardized difference)
Stable CVD	168 (80.1;255.9)	57.4 (9.9;109.4)		
HF			0.33 (0.003;0.656)	1.23 (0.14;2.32)

All $p < 0.0001$.
Adapted from [20,24].
CVD: Cardiovascular disease; HF: Heart failure.

Table 2. Comparisons on available meta-analyses evaluating the relationship between testosterone supplementation and CV.

	Calof *et al.* (2005) [25]		Haddad *et al.* (2007) [26]		Fernández-Balsells *et al.* (2010) [27]		Xu *et al.* (2013) [28]	
Number of trials included	19		6		51		27	
Number of patients analyzed	1084		308		2679		2944	
Inclusion criteria	Yes	No	Yes	No	Yes	No	Yes	No
Time restriction (> 12 weeks)	X			X		X	X	
Age restriction (≥ 45 years old)	X			X		X		X
All available RCTs reporting CV adverse events		X	X			X		X
CV analysis								
All CV events	X		X			X	X	
Serious adverse events (including MACE)		X		X		X	X	
MACE		X		X		X		X
AMI	X		X		X			X
Acute coronary syndrome	X			X		X		X
Coronary by-pass surgery	X			X	X			X
Stroke	X			X		X		X
New heart failure		X		X		X		X
Arrhythmias	X			X	X			X
CV mortality		X		X	X		X	

AMI: Acute myocardial infarction; CV: Cardiovascular; MACE: Major adverse cardiovascular events; RCTs: Randomized controlled trials.

the authors (E.M, G.C), and conflicts were resolved by the third investigator (G.R). The authors did not employ search software. They hand-searched bibliographies of retrieved papers for additional references. The principal source of information was derived from published articles; if data were missing from publication, an attempt at retrieval was made through clinicaltrials.gov website.

6.2 Study selection

The authors included all RCTs enrolling men and comparing the effect of TS versus placebo on different end points giving CV-related events by study arm without any arbitrary restriction, even if CV events were not the principal end points (**Figure 1**; **Tables 3**,**4** and **5**) [31-42,44-102]. Studies not specifically stating the occurrence or absence of CV-related events were excluded from the analysis (**Table 6**) [103-124]. Studies using androgens other than TS as well as studies with simultaneous treatment with other hormones and drugs were excluded, unless there was a clearly defined treatment arm that received only T treatment. In addition, since phosphodiesterase type 5 inhibitors (PDE5s) have been reported to play a possible positive influence on CV outcomes [125-127], RCTs evaluating the effect of TS as an add-on to PDE5i were excluded from the analysis (**Table 6**) [128-131].

6.3 Outcome

The principal outcome of this analysis was the effect of TS, as compared with placebo, on the incidence of a new major adverse cardiovascular event (MACE). MACE was defined as the composite of CV death, non-fatal acute myocardial infarction (AMI) and stroke, and acute coronary syndromes and/or HF reported as serious adverse events (**Table 4**) [29].

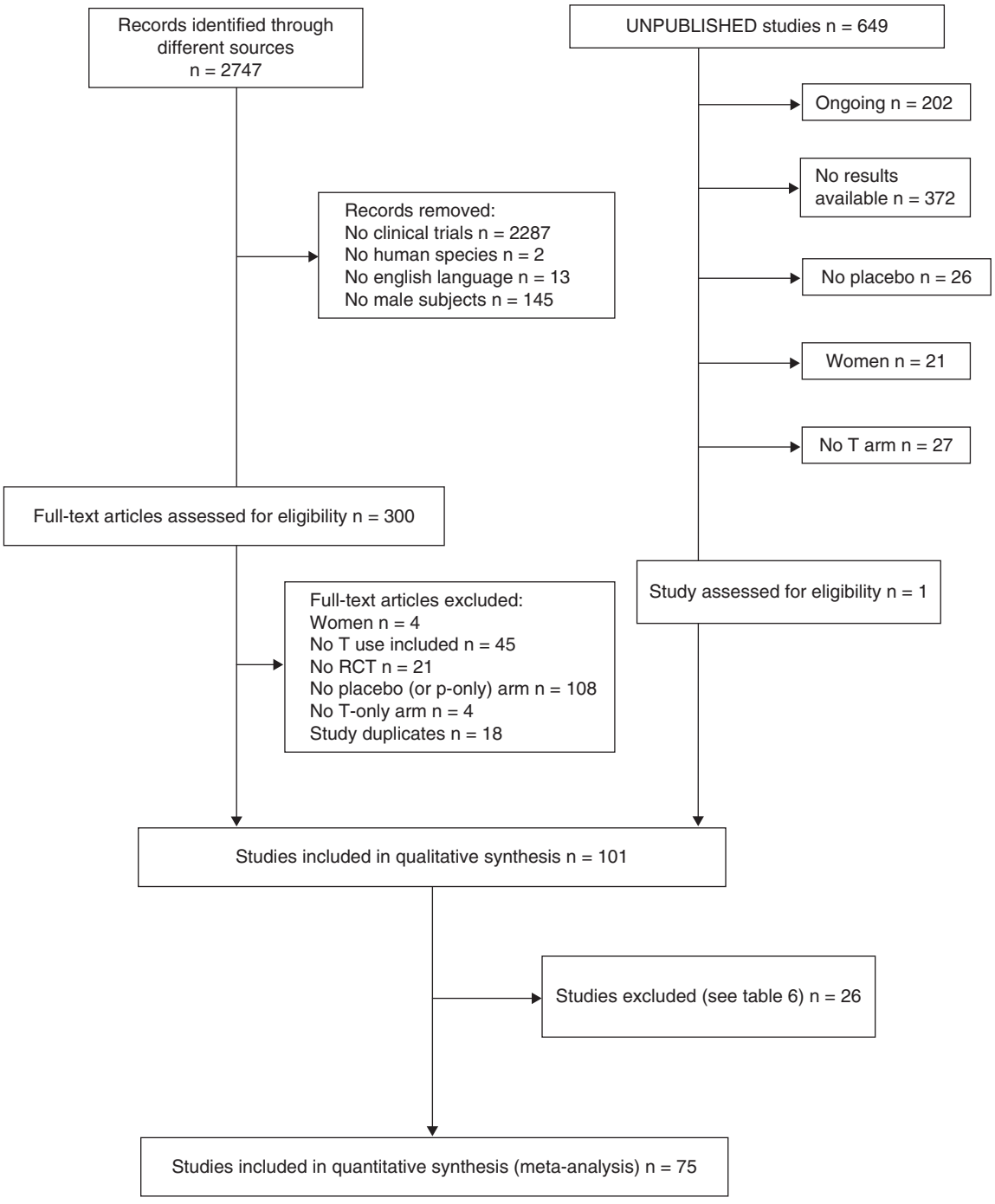


Figure 1. Trial flow diagram.
RCT: Randomized clinical trials; T: Testosterone.

Secondary outcomes included all CV-related events defined as anything reported as such by the authors, that is, events reported as cardiac disorders, CV complaints, CV events, vascular disorders, cardiac or CV, or when the event description fell within the International Statistical Classification of Disease (ICD) version 10 chapter IX (I00 – I99) (**Table 4**).

6.4 Quality assessment

The quality of RCTs was assessed using the Cochrane criteria [132]. In addition, the authors also employed the GRADE approach to rate the quality of evidence proposed by Jadad *et al.* [133] (**Table 5**). In particular, the following criteria were evaluated: how the randomization sequence was

Table 3. Characteristics of the randomized, placebo-controlled clinical studies included in the meta-analysis.

Study (ref.)	No. of patients (T/placebo)	Trial duration (weeks)	Age (years)	Comorbidities	Baseline total T (nmol/l)	T levels	Dose	Funding
Copenhagen Study Group (1986) [31]	134/87	112	53	Alcoholic cirrhosis*	NR	Mixed	Micronized T 600 mg/day	No
Drinka *et al.* (1995) [32]	8/10	26	NR	Institutionalized men*	NR	< 12 nM	TE 150 mg/70 kg/2 weeks	No
Aydin *et al.* (1996) [33]	20/18	8	38.9	Elderly men	NR	Eugonadal	TU 120 mg/day	No
Hall *et al.* (1996) [34]	35/35	39	60.8	Rheumatoid arthritis	15.9	Mixed	TE 250 mg/month	Yes
Coodley and Coodley (1997) [35]	17/18	13	NR	HIV	NR	Mixed	TC 200 mg/2 weeks	Yes
Sih *et al.* (1997) [36]	17/15	26	66.4	Elderly men	9.1	< 12 nM	TC 200 mg/2 weeks	No
Bhasin *et al.* (1998) [37]	20/21	12	NR	HIV	8	< 12 nM	T patch 5 mg/day	Yes
Grinspoon *et al.* (1998) [38]	26/25	26	42	HIV	10.7	Mixed	TE 300 mg/3 weeks	Yes
Clague *et al.* (1999) [39]	7/7	12	66.7	Elderly men	11.5	Mixed	TE 200 mg/2 weeks	Yes
Snyder *et al.* (1999) [40]	54/54	156	73.1	Elderly men	12.6	Mixed	T patch 6 mg/day	No
Bhasin *et al.* (2000) [41]	17/14	16	41.3	HIV	6.7	< 12 nM	TE 100 mg/weeks	No
English *et al.* (2000) [42]	25/25	12	62	Chronic stable angina	12.9	Mixed	T patch 5 mg/day	No
Grinspoon *et al.* (2000) [43]	27/27	12	41,9	HIV	22.8	Eugonadal	TE 200 mg/week	No
Rabkin *et al.* (2000) [44]	39/35	6	39	HIV	13.2	Mixed	TC 200 mg/2 weeks	Yes
Münzer *et al.* (2001) [45]	21/17	26	70	Elderly men	13.8	Eugonadal	TE 100 mg/2 weeks	No
Howell *et al.* (2001) [46]	16/19	26	40.9	Cytotoxic chemotherapy*	13.3	Mixed	T patch 2.5 – 5 mg/day	Yes
Seidman *et al.* (2001) [47]	13/17	6	51.4	Major depressive disorder	9.1	< 12 nM	TE 200 mg/week	No
Simon *et al.* (2001) [48]	6/6	12	54.1	LOH	8.7	< 12 nM	TG 125 mg/day	No
Amory *et al.* (2002) [49]	12/13	4	70.4	Elderly men	12.8	Mixed	TE 600 mg/week	No
Ferrando *et al.* (2002) [50]	7/5	26	67.6	Elderly men	11.3	Mixed	TE 100 mg/week	No
Pope *et al.* (2003) [51]	12/20	8	49.2	Refractory depression	NR	< 12 nM	TG 100 mg/day	Yes
Steidle *et al.* (2003) [52]	106/99	12	56.8	Sexual dysfunction	8	< 12 nM	TG 100 mg/day	Yes
Tan and Pu (2003) [53]	5/5	52	70.7	Alzheimer's disease	NR	< 8 nM	TE 200 mg/2 weeks	No
Amory *et al.* (2004) [54]	24/24	156	71	Elderly men	10.2	< 12 nM	TE 200 mg/2 weeks	No
Cavallini *et al.* (2004) [55]	40/45	52	63.5	Elderly men	10.2	< 12 nM	TU 160 mg/day	No
Kenny *et al.* (2004) [56]	6/5	12	79.6	Mild cognitive loss	13.8	Mixed	TE 200 mg/3 weeks	No
Malkin *et al.* (2004) [57]	5/5	4	60.8	Ischemic heart disease	4.2	Eugonadal	Sustanon 100 mg/2 weeks	No
Malkin *et al.* (2004) [58]	29/29	4	61.6	Elderly men	4.4	< 8 nM	Sustanon 100 mg/2 weeks	No
Rabkin *et al.* (2004) [59]	38/39	8	41	HIV	20.4	Mixed	TC 400 mg/2 weeks	Yes
Svartberg *et al.* (2004) [60]	15/14	26	66	COPD	21.1	Eugonadal	TE 250 mg/month	No
Seidman *et al.* (2005) [61]	13/13	6	46.4	Refractory depression	14.3	< 12 nM	TE 200 mg/week	No
Sullivan *et al.* (2005) [62]	37/34	12	78.2	Elderly frail men*	10.7	Mixed	TE 100 mg/week	No
Brockenbrough *et al.* (2006) [63]	19/21	26	55.8	Dialysis subjects*	7.2	< 12 nM	TG 100 mg/day	Yes
Giannoulis *et al.* (2006) [64]	23/20	26	69.9	Elderly men	16	Mixed	T patch 5 mg/day	No
Gold *et al.* (2006) [65]	66/80	12	39.9	HIV	25.7	Eugonadal	Sustanon250 mg/2 weeks	Yes
Kapoor *et al.* (2006) [66]	27/27	26	64	T2M	8.6	< 8 nM	Sustanon 200 mg/2 weeks	No
Katznelson *et al.* (2006) [67]	17/17	12	72	Elderly men	14.1	< 12 nM	T patch 5 mg/day	Yes
Lu *et al.* (2006) [68]‡	9/9	24	69.8	Mild Alzheimer's disease	12.2	Mixed	TG 75 mg/day	Yes

*Considered as frail men.

‡Subjects with Alzheimer's disease.

A: Adequate; BPH: Benign prostatic hyperplasia; COPD: Chronic obstructive pulmonary diseases; LOH: Late onset hypogonadism; MetS: Metabolic syndrome; NR: Not reported; OSA: Obstructive sleep apnea; T: Testosterone; T2DM: Type 2 diabetes mellitus; TC: Testosterone cypionate; TE: Testosterone enanthate; TG: Testosterone gel; TU: Testosterone undecanoate.

Table 3. Characteristics of the randomized, placebo-controlled clinical studies included in the meta-analysis (continued).

Study (ref.)	No. of patients (T/placebo)	Trial duration (weeks)	Age (years)	Comorbidities	Baseline total T (nmol/l)	T levels	Dose	Funding
Lu *et al.* (2006) [68]	14/15	24	62.4	Elderly men	13	Mixed	TG 75 mg/day	Yes
Malkin *et al.* (2006) [69]	37/39	52	64	Heart failure	13	Mixed	T patch 5 mg/day	No
Marks *et al.* (2006) [70]	22/22	26	69	Elderly men	8.1	< 12 nM	TE 150 mg/2 weeks	Yes
Merza *et al.* (2006) [71]	20/19	26	61.4	Elderly men	8	< 12 nM	T patch 5 mg/day	Yes
Nair *et al.* (2006) [72]	30/32	104	66.7	Elderly men	13	Mixed	T patch 5 mg/day	No
Okun *et al.* (2006) [73]	15/15	26	68.3	Parkinson's disease	11.1	Mixed	TE 200 mg/week	No
Bhasin *et al.* (2007) [74]	44/44	24	47	HIV	13.9	Mixed	TG 100 mg/day	No
Chiang *et al.* (2007) [75]	20/18	26	52	Elderly men	8.1	< 12 nM	TG 50 mg/day	Yes
Agledahl *et al.* (2008) [76]	13/13	52	69.1	Elderly men	8.4	< 12 nM	TU 1000 mg/12 weeks	Yes
Allan *et al.* (2008) [77]	31/31	52	63.3	Elderly men	14.1	Mixed	T patch 5 mg/day	Yes
Basurto *et al.* (2008) [78]	25/23	52	63.2	Elderly men	10.5	< 12 nM	TE250 mg/3 weeks	No
Emmelot-Vonk *et al.* (2008) [79]	120/117	26	67.3	Elderly men	10.7	Mixed	TU160 mg/day	No
Knapp *et al.* (2008) [80]	30/31	17	43.2	HIV	14.6	Mixed	TE300 mg/week	No
Svartberg *et al.* (2008) [81]	19/19	52	69	Elderly men	8.3	< 12 nM	TU 1000 mg/12 weeks	Yes
Caminiti *et al.* (2009) [82]	35/35	12	70	Heart failure	7.53	Mixed	TU 1000 mg/12 weeks	Yes
Chiang *et al.* (2009) [83]	20/20	13	NR	Erectile dysfunction	6.2	Mixed	TG 50 mg/day	No
Chapman *et al.* (2009) [84]	6/6	52	76	Elderly frail men*	20.3	Mixed	TU160 mg/day	Yes
Legros *et al.* (2009) [85]	237/79	52	58.7	Elderly men	12.8	Mixed	TU 80 – 240 mg/day	Yes
Mathur *et al.* (2009) [86]	7/6	52	64.8	Stable chronic angina	9.9	< 12 nM	TU 1000 mg/12 weeks	Yes
Seidman *et al.* (2009) [87]	13/10	6	50.6	Dysthymia	11.6	Mixed	TC200 mg/10 days	No
Shores *et al.* (2009) [88]	17/16	12	59.3	Dysthymia	10.1	Mixed	TG 75 mg/day	Yes
Aversa *et al.* (2010) [89]	40/10	104	57.8	MetS and/or T2DM	8.5	< 12 nM	TU 1000 mg/12 weeks	No
Aversa *et al.* (2010) [90]	42/10	52	57.2	MetS and/or T2DM	NR	< 12 nM	TU 160 mg/day/TU 1000 mg/12 weeks	No
Basaria *et al.* (2010) [11]	106/103	26	74	Elderly frail men*	8.3	< 12 nM	TG 100 mg/day	Yes
Gopal *et al.* (2010) [91]	11/11	26	44.2	T2DM	10.1	< 12 nM	TC 200 mg/2 weeks	No
Kalinchenko *et al.* (2010) [92]	113/71	30	52.1	MetS	7	< 12 nM	TU 1000 mg/12 weeks	Yes
Srinivas-Shankar *et al.* (2010) [93]	136/138	26	73.8	Elderly frail men*	11	Mixed	TG 50 mg/day	Yes
Amiaz *et al.* (2011) [94]	50/50	6	51.1	Major depressive disorder	11.8	Mixed	TG 25 – 100 mg/day	No
Ho *et al.* (2011) [95]	60/60	48	53.2	Elderly men	9	Mixed	TU 1000 mg/12 weeks	Yes
Jones *et al.* (2011) [96]	108/112	52	59.9	MetS and/or T2DM	9.5	Mixed	TG 60 mg/day	Yes
Kaufman *et al.* (2011) [97]	234/40	26	53.9	Elderly men	9.7	< 12 nM	TG 12.5 – 50 mg/day	Yes
Hoyos *et al.* (2012) [98]	33/34	18	48.5	Obese with OSA	13.3	Mixed	TU 1000 mg/12 weeks	Yes
Behre *et al.* (2012) [99]	183/179	48	62	Elderly men	10.5	Mixed	TG 50 – 75 mg/day	Yes
Hackett *et al.* (2013) [100]	92/98	30	61.6	T2DM	9	< 12 nM	TU 1000 mg/12 weeks	Yes
Hildreth *et al.* (2013) [101]	96/47	52	66.5	Elderly men	10.2	< 12 nM	TG 10 g/day	Yes
Maggio *et al.* (2013) [102]	43/24	156	71.8	Elderly men	13.4	Mixed	T patch 6 mg/day	No
NCT00957528	9/8	20	69.6	Elderly men	NA	Mixed	TE 100 mg/week	Yes

*Considered as frail men.

‡Subjects with Alzheimer's disease.

A: Adequate; BPH: Benign prostatic hyperplasia; COPD: Chronic obstructive pulmonary diseases; LOH: Late onset hypogonadism; MetS: Metabolic syndrome; NR: Not reported; OSA: Obstructive sleep apnea; T: Testosterone; T2DM: Type 2 diabetes mellitus; TC: Testosterone cypionate; TE: Testosterone enanthate; TG: Testosterone gel; TU: Testosterone undecanoate.

Table 4. Cardiovascular (CV) outcomes of the randomized, placebo-controlled clinical studies included in the meta-analysis.

First author (year)	All CV events (T/P)	MACE (T/P)	AMI (T/P)	ACS (T/P)	CBPS (T/P)	Stroke (T/P)	HF (T/P)	Arrythmias (T/P)	CV mortality (T/P)
Copenhagen Study Group (1986) [31]	16/5	1/0	1/0	1/0	0/0	0/0	0/0	0/0	1/0
Drinka *et al.* (1995) [32]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Aydin *et al.* (1996) [33]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Hall *et al.* (1996) [34]	0/2	0/1	0/0	0/0	0/0	0/1	0/0	0/0	0/0
Coodley *et al.* (1997) [35]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Sih *et al.* (1997) [36]	1/1	1/1	0/0	0/0	0/0	0/1	1/0	1/0	0/0
Bhasin *et al.* (1998) [37]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Grinspoon *et al.* (1998) [38]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Clague *et al.* (1999) [39]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Snyder *et al.* (1999) [40]	9/5	2/1	2/1	2/1	2/2	0/0	0/0	3/1	0/0
Bhasin *et al.* (2000) [41]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
English *et al.* (2000) [42]	2/0	1/0	1/0	1/0	0/0	0/0	0/0	0/0	0/0
Grinspoon *et al.* (2000) [43]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Rabkin *et al.* (2000) [44]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Munzer *et al.* (2001) [45]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Howell *et al.* (2001) [46]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Seidman *et al.* (2001) [47]	0/1	0/1	0/1	0/0	0/0	0/0	0/0	0/0	0/0
Simon *et al.* (2001) [48]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Amory *et al.* (2002) [49]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Ferrando *et al.* (2002) [50]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Pope *et al.* (2003) [51]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Steidle *et al.* (2003) [52]	2/0	1/0	0/0	1/0	0/0	0/0	0/0	0/0	0/0
Tan *et al.* (2003) [53]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Amory *et al.* (2004) [54]	2/0	1/0	0/0	0/0	0/0	1/0	0/0	1/0	0/0
Cavallini *et al.* (2004) [55]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Kenny *et al.* (2004) [56]	0/1	0/1	0/0	0/0	0/0	0/1	0/0	0/0	0/0
Malkin *et al.* (2004) [57]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Malkin *et al.* (2004) [58]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Rabkin *et al.* (2004) [59]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Svartberg *et al.* (2004) [60]	0/1	0/1	0/1	0/1	0/0	0/0	0/0	0/0	0/1
Seidman *et al.* (2005) [61]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Sullivan *et al.* (2005) [62]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Brockenbrough *et al.* (2006) [63]	9/9	3/1	0/0	NR	0/0	0/1	0/0	NR	3/1
Giannoulis *et al.* (2006) [64]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Gold *et al.* (2006) [65]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Kapoor *et al.* (2006) [66]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Katznelson *et al.* (2006) [67]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Lu *et al.* (2006) [68]*	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Lu *et al.* (2006) [68]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Malkin *et al.* (2006) [69]	5/6	2/1	0/0	1/0	0/0	1/0	0/0	0/2	0/1

*Subjects with Alzheimer's disease.

ACS: Acute coronary syndrome; AMI: Acute myocardial infarction; CBPS: Coronary by-pass surgery; HF: Heart failure; MACE: Major adverse cardiovascular events; NR: Not reported; P: Placebo; T: Testosterone.

Table 4. Cardiovascular (CV) outcomes of the randomized, placebo-controlled clinical studies included in the meta-analysis (continued).

First author (year)	All CV events (T/P)	MACE (T/P)	AMI (T/P)	ACS (T/P)	CBPS (T/P)	Stroke (T/P)	HF (T/P)	Arrythmias (T/P)	CV mortality (T/P)
Marks *et al.* (2006) [70]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Merza *et al.* (2006) [71]	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Nair *et al.* (2006) [72]	7/6	2/0	0/0	2/0	3/0	0/0	0/0	0/0	0/0
Okun *et al.* (2006) [73]	1/2	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0
Bhasin *et al.* (2007) [74]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Chiang *et al.* (2007) [75]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Agledahl *et al.* (2008) [76]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Allan *et al.* (2008) [77]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Basurto *et al.* (2008) [78]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Emmelot-Vonk *et al.* (2008) [79]	7/3	NR	NR	NR	NR	NR	NR	NR	0/0
Knapp *et al.* (2008) [80]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Svartberg *et al.* (2008) [81]	1/0	1/0	0/0	0/0	0/0	0/0	0/0	1/0	1/0
Caminiti *et al.* (2009) [82]	2/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Chiang *et al.* (2009) [83]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Chapman *et al.* (2009) [84]	1/1	1/1	1/1	1/1	0/0	0/0	0/0	0/0	1/0
Legros *et al.* (2009) [85]	1/0	1/0	0/0	0/0	0/0	0/0	0/0	1/0	1/0
Mathur *et al.* (2009) [86]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Seidman *et al.* (2009) [87]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Shores *et al.* (2009) [88]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Aversa *et al.* (2010) [89]	0/1	0/1	0/1	0/1	0/0	0/0	0/0	0/0	0/0
Aversa *et al.* (2010) [90]	0/1	0/1	0/1	0/1	0/0	0/0	0/0	0/0	0/0
Basaria *et al.* (2010) [11]	25/5	6/0	3/0	5/0	1/0	1/0	0/0	5/2	1/0
Gopal *et al.* (2010) [91]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Kalinchenko *et al.* (2010) [92]	0/2	0/1	0/1	0/0	0/0	0/0	0/0	0/0	0/2
Srinivas-Shankar *et al.* (2010) [93]	5/2	2/2	0/1	0/0	0/0	0/0	1/0	0/0	1/1
Amiaz *et al.* (2011) [94]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Ho *et al.* (2011) [95]	2/2	1/1	1/1	1/1	0/0	0/0	0/0	0/0	1/1
Jones *et al.* (2011) [96]	5/12	1/2	1/2	1/2	0/0	0/0	0/0	0/0	0/1
Kaufman *et al.* (2011) [97]	17/2	2/0	1/0	1/0	0/0	0/0	1/0	0/0	0/0
Hoyos *et al.* (2012) [98]	1/0	NR	NR	NR	NR	NR	NR	NR	0/0
Behre *et al.* (2012) [99]	NR	1/0	NR	NR	NR	NR	NR	NR	1/0
Hackett *et al.* (2013) [100]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Hildreth *et al.* (2013) [101]	4/11	1/3	0/0	1/3	0/0	0/0	0/0	0/4	0/0
Maggio *et al.* (2013) [102]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
NCT00957528	1/1	0/0	NR	NR	NR	NR	NR	NR	NR

*Subjects with Alzheimer's disease.

ACS: Acute coronary syndrome; AMI: Acute myocardial infarction; CBPS: Coronary by-pass surgery; HF: Heart failure; MACE: Major adverse cardiovascular events; NR: Not reported; P: Placebo; T: Testosterone.

Table 5. Characteristics and outcomes of the randomized, placebo-controlled clinical studies included in the meta-analysis.

Study (ref.)	Design	Randomization	Blinding	Drop-out	Jadad score	ITT	Eligibility criteria listed	Definition of MACE predetermined	Table for MACE occurred in study arms
Copenhagen Study Group (1986) [31]	Parallel	A	A	A	5	Yes	Yes	Yes	Yes
Drinka *et al.* (1995) [32]	Parallel	A	NA	A	2	Yes	No	NR	No
Aydin *et al.* (1996) [33]	Parallel	A	NA	NA	1	No	No	NR	No
Hall *et al.* (1996) [34]	Parallel	A	A	A	3	No	Yes	NR	No
Coodley *et al.* (1997) [35]	Cross-over	A	A	A	3	No	Yes	NR	No
Sih *et al.* (1997) [36]	Parallel	A	NA	A	3	No	Yes	NR	No
Bhasin *et al.* (1998) [37]	Parallel	A	A	A	3	No	Yes	NR	No
Grinspoon *et al.* (1998) [38]	Parallel	A	A	A	5	Yes	Yes	NR	No
Clague *et al.* (1999) [39]	Parallel	A	A	A	4	No	No	NR	No
Snyder *et al.* (1999) [40]	Parallel	A	A	A	4	Yes	Yes	NR	Yes
Bhasin *et al.* (2000) [41]	Parallel	A	A	A	4	Yes	Yes	NR	No
English *et al.* (2000) [42]	Parallel	A	A	A	3	No	Yes	NR	No
Grinspoon *et al.* (2000) [43]	Parallel	A	A	A	5	No	Yes	NR	No
Rabkin *et al.* (2000) [44]	Parallel	A	A	A	5	No	Yes	NR	No
Munzer *et al.* (2001) [45]	Parallel	A	A	A	4	No	Yes	NR	No
Howell *et al.* (2001) [46]	Parallel	A	NA	A	2	No	Yes	NR	No
Seidman *et al.* (2001) [47]	Parallel	A	A	A	4	No	Yes	NR	No
Simon *et al.* (2001) [48]	Parallel	A	A	NA	3	No	Yes	NR	No
Amory *et al.* (2002) [49]	Parallel	A	A	A	4	No	Yes	NR	No
Ferrando *et al.* (2002) [50]	Parallel	A	A	NA	2	No	Yes	NR	No
Pope *et al.* (2003) [51]	Parallel	A	NA	A	3	Yes	Yes	NR	No
Steidle *et al.* (2003) [52]	Parallel	A	NA	NA	2	NR	Yes	NR	No
Tan *et al.* (2003) [53]	Parallel	A	NA	NA	1	No	No	NR	No
Amory *et al.* (2004) [54]	Parallel	A	A	A	4	Yes	Yes	NR	No
Cavallini *et al.* (2004) [55]	Parallel	A	A	A	3	No	Yes	NR	No
Kenny *et al.* (2004) [56]	Parallel	A	A	A	3	No	Yes	NR	No
Malkin *et al.* (2004) [57]	Cross-over	A	NA	A	3	No	Yes	NR	No
Malkin *et al.* (2004) [58]	Cross-over	A	NA	A	3	No	Yes	NR	No
Rabkin *et al.* (2004) [59]	Parallel	A	A	A	5	Yes	Yes	NR	No
Svartberg *et al.* (2004) [60]	Parallel	A	A	A	3	No	No	NR	No
Seidman *et al.* (2005) [61]	Parallel	A	A	A	5	Yes	Yes	NR	No
Sullivan *et al.* (2005) [62]	Parallel	A	A	A	3	Yes	Yes	NR	No
Brockenbrough *et al.* (2006) [63]	Parallel	A	A	A	5	Yes	Yes	NR	Yes
Giannoulis *et al.* (2006) [64]	Parallel	A	A	A	5	Yes	Yes	NR	No
Gold *et al.* (2006) [65]	Parallel	A	A	A	5	Yes	Yes	NR	No
Kapoor *et al.* (2006) [66]	Cross-over	A	A	A	5	No	Yes	NR	No
Katznelson *et al.* (2006) [67]	Parallel	A	A	A	4	Yes	Yes	NR	No
Lu *et al.* (2006) [68]*	Parallel	A	A	A	4	Yes	Yes	NR	No
Lu *et al.* (2006) [68]	Parallel	A	A	A	4	Yes	Yes	NR	No

*Subjects with Alzheimer's disease.

A: Adequately described; ITT: Intention to treat; MACE: Major adverse cardiovascular events; NA: Non-adequately described; NR: Not reported.

Table 5. Characteristics and outcomes of the randomized, placebo-controlled clinical studies included in the meta-analysis (continued).

Study (ref.)	Design	Randomization	Blinding	Drop-out	Jadad score	ITT	Eligibility criteria listed	Definition of MACE predetermined	Table for MACE occurred in study arms
Malkin *et al.* (2006) [69]	Parallel	A	A	A	4	Yes	Yes	NR	Yes
Marks *et al.* (2006) [70]	Parallel	A	A	A	3	No	Yes	NR	No
Merza *et al.* (2006) [71]	Parallel	A	A	A	3	No	Yes	NR	No
Nair *et al.* (2006) [72]	Parallel	A	A	NA	3	Yes	Yes	NR	Yes
Okun *et al.* (2006) [73]	Parallel	A	A	NA	3	NR	Yes	NR	Yes
Bhasin *et al.* (2007) [74]	Parallel	A	A	A	3	Yes	Yes	NR	No
Chiang *et al.* (2007) [75]	Parallel	A	A	A	3	Yes	Yes	NR	Yes
Agledahl *et al.* (2008) [76]	Parallel	A	A	A	4	No	Yes	NR	No
Allan *et al.* (2008) [77]	Parallel	A	A	A	4	Yes	Yes	NR	No
Basurto *et al.* (2008) [78]	Parallel	A	A	A	5	Yes	Yes	NR	No
Emmelot-Vonk *et al.* (2008) [79]	Parallel	A	A	A	5	Yes	Yes	Yes	Yes
Knapp *et al.* (2008) [80]	Parallel	A	A	A	5	Yes	Yes	NR	No
Svartberg *et al.* (2008) [81]	Parallel	A	A	A	4	No	Yes	NR	No
Caminiti *et al.* (2009) [82]	Parallel	A	A	A	3	No	Yes	NR	No
Chiang *et al.* (2009) [83]	Parallel	A	A	A	4	No	No	NR	No
Chapman *et al.* (2009) [84]	Parallel	A	NA	A	2	No	Yes	NR	No
Legros *et al.* (2009) [85]	Parallel	A	A	A	5	No	Yes	NR	No
Mathur *et al.* (2009) [86]	Parallel	A	A	NA	4	Yes	Yes	NR	No
Seidman *et al.* (2009) [87]	Parallel	A	A	A	5	Yes	Yes	NR	No
Shores *et al.* (2009) [88]	Parallel	A	A	A	4	Yes	Yes	NR	No
Aversa *et al.* (2010) [89]	Parallel	A	A	NA	2	No	Yes	NR	No
Aversa *et al.* (2010) [90]	Parallel	A	A	NA	3	No	Yes	NR	No
Basaria *et al.* (2010) [11]	Parallel	A	A	A	3	Yes	Yes	Yes	Yes
Gopal *et al.* (2010) [91]	Cross-over	A	A	NA	4	No	Yes	NR	No
Kalinchenko *et al.* (2010) [92]	Parallel	A	A	A	4	Yes	Yes	NR	No
Srinivas-Shankar *et al.* (2010) [93]	Parallel	A	NA	A	3	Yes	Yes	NR	No
Amiaz *et al.* (2011) [94]	Parallel	A	A	NA	3	No	Yes	NR	No
Ho *et al.* (2011) [95]	Parallel	A	A	A	3	No	Yes	NR	No
Jones *et al.* (2011) [96]	Parallel	A	A	NA	3	Yes	Yes	NR	No
Kaufman *et al.* (2011) [97]	Parallel	A	A	A	5	No	Yes	NR	Yes
Hoyos *et al.* (2012) [98]	Parallel	A	A	A	5	Yes	Yes	NR	Yes
Behre *et al.* (2012) [99]	Parallel	A	A	A	5	Yes	Yes	NR	No
Hackett *et al.* (2013) [100]	Parallel	A	A	A	5	Yes	Yes	NR	No
Hildreth *et al.* (2013) [101]	Parallel	A	A	NA	4	Yes	Yes	NR	Yes
Maggio *et al.* (2013) [102]	Parallel	A	A	NA	3	No	Yes	NR	No
NCT00957528	Parallel	A	A	NA	2	Yes	Yes	NR	No

*Subjects with Alzheimer's disease.

A: Adequately described; ITT: Intention to treat; MACE: Major adverse cardiovascular events; NA: Non-adequately described; NR: Not reported.

Table 6. Studies that met inclusion criteria but did not provide data for meta-analysis.

First author, year (ref.)	Brief description of the study and main conclusions
Johnsen *et al.* (1974) [103]	Cross-over RCT on the clinical effectiveness of oral testosterone replacement (400 mg/day) in hypogonadal subjects. Whether potentially adverse events occurred or not is not explicitly mentioned in the full-text article
Benkert *et al.* (1979) [104]	RCT on the effects of testosterone undecanoate administration (120 mg/day) for 8 weeks on sexual potency in eugonadal subjects with erectile dysfunction. Whether potentially adverse events occurred or not is not explicitly mentioned in the full-text article
Davidson *et al.* (1979) [105]	Cross-over RCT on the effects of testosterone enanthate replacement (100 or 400 mg every 4 weeks) for 20 weeks on sexual behavior in hypogonadal subjects. Whether potentially adverse events occurred or not is not explicitly mentioned in the full-text article
Skakkebaek *et al.* (1981) [106]	Cross-over RCT on the effects of testosterone undecanoate replacement (160 mg/day) for 8 weeks on sexual interest and behavior in hypogonadal subjects. Whether potentially adverse events occurred or not is not explicitly mentioned in the full-text article
Bancroft and Wu (1983) [107]	Cross-over RCT on the effects of testosterone undecanoate replacement (160 or 240 mg/day) for 8 weeks on erections in response to erotic films and fantasies in hypogonadal subjects. Whether potentially adverse events occurred or not is not explicitly mentioned in the full-text article
Kwan *et al.* (1983) [108]	Cross-over RCT on the effects of testosterone enanthate replacement (200 or 400 mg/month) for 12 weeks on sexual function in hypogonadal subjects. Whether potentially adverse events occurred or not is not explicitly mentioned in the full-text article
O'Carroll and Bancroft (1984) [109]	Cross-over RCT on the effects of mixed testosterone esters administration (250 mg/2 weeks) for 12 weeks in eugonadal subjects with loss of sexual interest or erectile dysfunction. Whether potentially adverse events occurred or not is not explicitly mentioned in the full-text article
Nankin *et al.* (1986) [110]	Cross-over RCT on the effects of testosterone cypionate replacement (200 mg/2 weeks) for 12 weeks on sexual function in hypogonadal men with erectile dysfunction. Whether potentially adverse events occurred or not is not explicitly mentioned in the full-text article
Gluud *et al.* (1988) [111]	RCT on the effects of micronized oral testosterone administration (600 mg/day) for 112 weeks on sexual function in subjects with alcoholic cirrhosis. Whether potentially adverse events occurred or not is not explicitly mentioned in the full-text article
Carani *et al.* (1990) [112]	Cross-over RCT on the effects of testosterone undecanoate replacement (160 mg/day) for 12 weeks on sexual function in mild hypogonadal men with erectile dysfunction. Whether potentially adverse events occurred or not is not explicitly mentioned in the full-text article
Marin *et al.* (1992) [113]	RCT of the effects on testosterone undecanoate replacement (160 mg/day) for 32 weeks on body composition and metabolism in middle-aged obese men. Whether potentially adverse events occurred or not is not explicitly mentioned in the full-text article
Clopper *et al.* (1993) [114]	Cross-over RCT on the effects of testosterone enathate (200 mg/fortnightly) for 4 weeks on psychosexual behavior in hypopituitary subjects. Whether potentially adverse events occurred or not is not explicitly mentioned in the full-text article
Schiavi *et al.* (1997) [115]	Cross-over RCT on the effects of testosterone enanthate administration (200 mg/2 weeks) for 6 weeks on sexual behavior and mood in subjects with erectile dysfunction. Whether potentially adverse events occurred or not is not explicitly mentioned in the full-text article
Dobs *et al.* (1998) [116]	RCT on the pharmacokinetic characteristics and clinical effects of buccal testosterone replacement (10 mg/day) for 8 weeks in hypogonadal subjects. Whether potentially adverse events occurred or not is not explicitly mentioned in the full-text article
Anderson *et al.* (1999) [117]	RCT on the effects of testosterone enanthate administration (200 mg/week) for 8 weeks on sexuality and mood in eugonadal subjects. Whether potentially adverse events occurred or not is not explicitly mentioned in the full-text article
Wolf *et al.* (2000) [118]	RCT on the effects of a single testosterone enanthate administration (250 mg) on cognitive performance in elderly men. Whether potentially adverse events occurred or not is not explicitly mentioned in the full-text article
Kenny *et al.* (2002) [119]	RCT on the effects of transdermal testosterone replacement (2 – 2.5 mg/day) for 52 weeks on lipids and vascular reactivity in hypogonadal older men. The authors report that four subjects in the testosterone-arm and five subjects in the placebo-arm withdrew from the study for 'intercurrent illness', whose nature is not further specified
O' Connor *et al.* (2002) [120]	RCT on the effects of testosterone enanthate administration (200 mg weekly) for 8 weeks on self- and partner-reported aggression and mood in eugonadal men. Whether potentially adverse events occurred or not is not explicitly mentioned in the full-text article

CV: Cardiovascular; PDE5i: Phosphodiesterase type 5 inhibitor; RCT: Randomized clinical trials.

Table 6. Studies that met inclusion criteria but did not provide data for meta-analysis (continued).

First author, year (ref.)	Brief description of the study and main conclusions
Malkin *et al.* (2003) [121]	RCT on the effects of mixed testosterone esters administration (100 mg/2 weeks) for 12 weeks on TNF-α production in men with chronic heart failure. Whether potentially adverse events occurred or not is not explicitly mentioned in the full-text article
Haren *et al.* (2005) [122]	RCT of the effects of testosterone undecanoate replacement (160 mg/day) for 53 weeks on testosterone deficiency symptoms in symptomatic elderly males with low-normal gonadal status. Whether potentially adverse events occurred or not is not explicitly mentioned in the full-text article
Sheffield-Moore *et al.* (2011) [123]	RCT of the effects of testosterone enanthate administration (100 mg/month or every 2 months) for 20 weeks on body composition and muscle strength in older men. The authors mention that "adverse events were minimal and not limited to a specific treatment group" without specifying the nature of the events and the arm in which they occurred
Fredirksen *et al.* (2012) [124]	RCT of the effects of transdermal testosterone administration (50 or 100 mg/day) for 24 weeks on subcutaneous fat and adiponectin in aging men. Whether potentially adverse events occurred or not is not explicitly mentioned in the full-text article
Aversa *et al.* (2003) [128]	Cross-over RCT of the effects of testosterone patch (50 mg/daily) and sildenafil for 8 weeks on sexual function in hypogonadal men with erectile dysfunction. The use of PDE5i along with testosterone or placebo can influence CV outcome
Shabsigh *et al.* (2004) [129]	RCT of the effects of testosterone gel (50 mg/daily) and sildenafil for 12 weeks on sexual function in hypogonadal men with erectile dysfunction. The use of PDE5i along with testosterone or placebo can influence CV outcome
Buvat *et al.* (2011) [130]	RCT of the effects of testosterone gel (50 mg/daily) and tadalafil for 12 weeks on sexual function in hypogonadal men with erectile dysfunction. The use of PDE5i along with testosterone or placebo can influence CV outcome
Spitzer *et al.* (2012) [131]	RCT of the effects of testosterone gel (100 mg/daily) and sildenafil for 14 weeks on sexual function in hypogonadal men with erectile dysfunction. The use of PDE5i along with testosterone or placebo can influence CV outcome

CV: Cardiovascular; PDE5i: Phosphodiesterase type 5 inhibitor; RCT: Randomized clinical trials.

generated, how allocation was concealed, whether there were important imbalances at baseline, which groups were blinded (patients, caregivers, data collectors, outcome assessors, data analysts), what the loss to follow-up rate was (in the intervention and the control arm), whether the analyses were by intention to treat and how missing outcome data were dealt with. For each study, the authors also assessed how the population was selected, the duration and route of TS, the adequacy of study follow-up and the funding source. In particular, a trial was considered partially or completely supported by pharmaceutical companies when declared by the authors and/or when at least one author belongs to any industry company, as previously reported [9].

6.5 Statistical analysis

Heterogeneity on MACE was assessed by using I^2 statistics. Even when a low heterogeneity was detected, a random-effects model was applied, because the validity of tests of heterogeneity can be limited with a small number of component studies. To estimate possible publication or disclosure bias, the authors used funnel plots and the Begg adjusted rank correlation test [134,135]. However, because these tests have low statistical power when the number of trials is small, undetected bias may still be present. Mantel–Haenszel odds ratio with 95% CI (MH-OR) was calculated for all the adverse events defined above, on an intention-to-treat basis, excluding trials with zero events. A sensitivity analysis was performed with continuity corrections for trials with zero events. In addition, subanalysis considering the incidence of MACE according to baseline population characteristics was also performed. A meta-regression analysis was performed to test the effect of different parameters on TS-related MACE. All analyses were performed using comprehensive meta-analysis version 2, Biostat (Englewood, NJ, USA).

6.6 Clinical trial report results

Out of 2747 retrieved articles, 74 were included in the study (**Figure 1**) [11,31-42,44-102]. In addition, one completed but still unpublished study was also considered (NCT00957528, **Figure 1**). In particular, of those, 73, 71 and 71 reported information on MACE, AMI and stroke, respectively and 74 on CV mortality. In addition, 70 reported also information on acute coronary syndrome, 71 on arrhythmias, whereas 70 on by-pass coronary surgery or HF. The characteristics of the retrieved trials (including parameters on trial quality) and the number of events recorded are reported in **Tables 3**, **4** and **5**. Retrieved trials included 3016 and 2448 patients in TS and placebo groups, respectively; mean trial duration was 34.8 weeks.

The mean age, baseline T and body mass index of enrolled patients were 59.9 years, 11.2 nmol/l and 28.1 kg/m^2. TS was administered in different doses, formulations and cohorts; in

particular, 44 RCTs evaluated the effect of TS in a mixed population of hypogonadal/eugonadal subjects and 31 in hypogonadal patients (T below 12 nmol/l). In addition, 6 trials used oral T formulations, whereas 40 and 28 studies employed intramuscular and transdermal T preparation. Finally, in one RCT, both intramuscular and transdermal T preparations were used.

Of the 73 trials reporting information on MACE, 47 detected no events; therefore, the main analysis was performed on 26 trials. I^2 was 0.0 (p = 0.78). Funnel plot and Begg adjusted rank correlation test (Kendall's τ: -0.14; p = 0.33) suggested no major publication bias. The use of TS was not associated with any significant difference in the incidence of MACE with respect to placebo (MH-OR: 1.01 [0.57;1.77]; p = 0.98) (**Figure 2**). Meta-regression analysis showed no difference in the incidence of MACE according to baseline age, body mass index or level of T (S = 0.03 [-0.04;0.10]; p = 0.40, -0.07 [-0,29;0.14]; 0.51; -0.14 [-1.17;0.89]; p = 0.79]. A sensitivity analysis was performed with continuity correction, confirming the results of the main analysis (MH-OR: 0.98 [0.70;1.34]; p = 0.92). These results were confirmed applying Duval and Tweedie's trim and fill method which suggested no additional unpublished trials. In addition, similar results were confirmed when the individual MACE was analyzed separately (**Figure 3**). When separate analyses were performed according to the baseline population characteristics of the subjects enrolled, TS showed a protective role against incidence of MACE in subjects with metabolic disease, whereas no difference between TS and placebo was detected in other subpopulations including subjects with previous CVD or frail men (**Figure 4**). Finally, no difference in MACE incidence between TS and placebo was detected when the trials were categorized according to: i) baseline T levels; ii) the reported pharmaceutical industry support; or iii) trial duration > 12 weeks (**Figure 4**). Similar results were obtained when any CV-related events were considered (**Figure 5**).

6.7 Clinical trial report interpretation

The present systematic review and meta-analysis analysis comprehensively evaluates all the available data on the effect of TS on possible related CV events and/or mortality without any restriction. By meta-analyzing the largest number of studies collected so far, the authors did not observe any increase in CV risk associated with TS either when composite or single CV end points were considered. Our data are consistent with three previous meta-analyses [25-27], which considered both composite and individual CV end points, while they are in apparent disagreement with Xu *et al.* [28], which analyzed only all and serious composite CV end points documenting a significant increase in risk in the active treatment arm (**Table 2**). In contrast to Xu *et al.* [28], the authors did not observe any sponsorship bias, because no difference in the CV risk was found when the analysis was performed according to the presence or absence of drug company support. It should be stressed that the analysis by Xu *et al.* [28] included many events, which are not normally considered for the assessment of CV risk (e.g., peripheral edema, self-reported syncope etc.). An overly broad definition of CV end points, inappropriately reported as 'CV-related' by the investigators, increases the statistical power of the analysis, but, on the other hand, can be grossly misleading due to the heterogeneity and limited reliability of diagnostic criteria used to attribute these events as drug-related. Similarly, analyses of all CV events classified as 'serious' (which include all hospitalizations) has the limitation of including in the end points many events, which are investigator-driven, such as revascularization. The assessments of CV safety of any therapy should be based on the incidence of MACE, which are easier to detect and less controversial in diagnosis. Even for these, however, misclassification occurs, as a consequence of undefined screening procedures and diagnostic criteria, unless trials are specifically designed for CV outcomes. None of the reviewed studies were originally designed to address safety. Any results on CVD derived from trials without a formal adjudication of events should be considered with caution. In addition, the number of size of available trials is rather small, determining wide CI. Since OR for MACE with TS is 1.01 [0.57;1.77], the present data are sufficient only to exclude any increase of risk greater than 77%. Furthermore, the duration of the available trials is short. Therefore, although there is no clear sign of risk in the short term, the authors have no information on possible long-term effects. A further limitation is represented by incomplete reporting of the data on MACE in trials marginally designed for non-CV end points. Although statistical analyses did not suggest any relevant publication bias, the possibility of selective reporting cannot be excluded. However, it should be recognized that the use of MACE, instead of a broader definition of CV side effects, has the advantage of a clearer diagnostic definition, which is less dependent on investigators' subjective opinions. In fact, although criteria for diagnosis of myocardial infarction or stoke can also be questioned, such entities are much more clearly defined than 'peripheral edema' or 'self-reported syncope', which are included among the CV events in other analyses [11,28]. Interestingly, regulatory agencies assessing the safety of drugs require analyses of MACE and not of broadly defined CV side effects [29].

When the analysis was performed according to the baseline study population characteristic, the authors observed a possible protective role of TS in subjects with metabolic disease. This is not a surprise because, by meta-analyzing the available evidence, the authors previously demonstrated that TS is associated with an improvement of fat mass and glycometabolic control in subjects with type 2 diabetes (T2DM) and metabolic syndrome (MetS, [3,136],). Similar results have been more recently confirmed by other authors [137]. However, it should be recognized that the number of RCTs evaluating the effect of TS in subjects with metabolic disease is too limited to draw any conclusions.

Source	MH-OR	LL	UL	P	TS No. events	TS No. patients	Placebo No. events	Placebo No. patients
Copenhagen SG 1986 [31]	1.97	0.08	48.82	0.68	1	134	0	87
Hall *et al.*, 1996 [34]	0.32	0.01	8.23	0.49	0	35	1	35
Sih *et al.*, 1997 [36]	0.88	0.05	15.33	0.93	1	17	1	15
Snyder *et al.*, 1999 [40]	2.04	0.18	23.17	0.57	2	54	1	54
English *et al.*, 2000 [42]	3.12	0.12	80.39	0.49	1	25	0	25
Seidman *et al.*, 2001 [47]	0.41	0.02	10.83	0.59	0	13	1	17
Steidle *et al.*, 2003 [52]	2.83	0.11	70.27	0.53	1	106	0	99
Amory *et al.*, 2004 [54]	3.13	0.12	80.68	0.49	1	24	0	24
Kenny *et al.*, 2004 [56]	0.23	0.01	7.05	0.40	0	6	1	5
Svartberg *et al.*, 2004 [60]	0.29	0.01	7.74	0,46	0	15	1	14
Brockenbrough *et al.*, 2006 [63]	3.75	0.36	39.59	0.27	3	19	1	21
Malkin *et al.*, 2006 [69]	2.17	0.19	25.01	0.53	2	37	1	39
Nair *et al.*, 2006 [72]	5.70	0.26	123.78	0.27	2	30	0	32
Svartberg *et al.*, 2008 [81]	3.16	0.12	82.64	0.49	1	19	0	19
Chapman *et al.*, 2009 [84]	1.00	0.05	20.83	1.00	1	6	1	6
Legros *et al.*, 2009 [85]	1.01	0.04	25.01	1.00	1	237	0	79
Aversa *et al.*, 2010 [89]	0.08	0.00	2.07	0.13	0	40	1	10
Aversa *et al.*, 2010 [90]	0.07	0.00	1.97	0.12	0	42	1	10
Basaria *et al.*, 2010 [11]	13.39	0.74	240.78	0.08	6	106	0	103
Kalinchenko *et al.*, 2010 [92]	0.21	0.01	5.15	0.34	0	113	1	71
Srinivas-Shankar *et al.*, 2010 [93]	1.01	0.14	7.31	0.99	2	136	2	138
Ho *et al.*, 2011 [95]	1.00	0.06	16.37	1.00	1	60	1	60
Jones *et al.*, 2011 [96]	0.51	0.05	5.57	0.59	1	108	2	112
Kaufman *et al.*, 2011 [97]	0.87	0.04	18.48	0.93	2	234	0	40
Behre *et al.*, 2012 [99]	2.95	0.12	72.91	0.51	1	183	0	179
Hildreth *et al.*, 2013 [100]	0.15	0.02	1.53	0.11	1	96	3	47
Overall	**1.01**	**0.57**	**1.77**	**0.98**	**31**	**1895**	**20**	**1341**

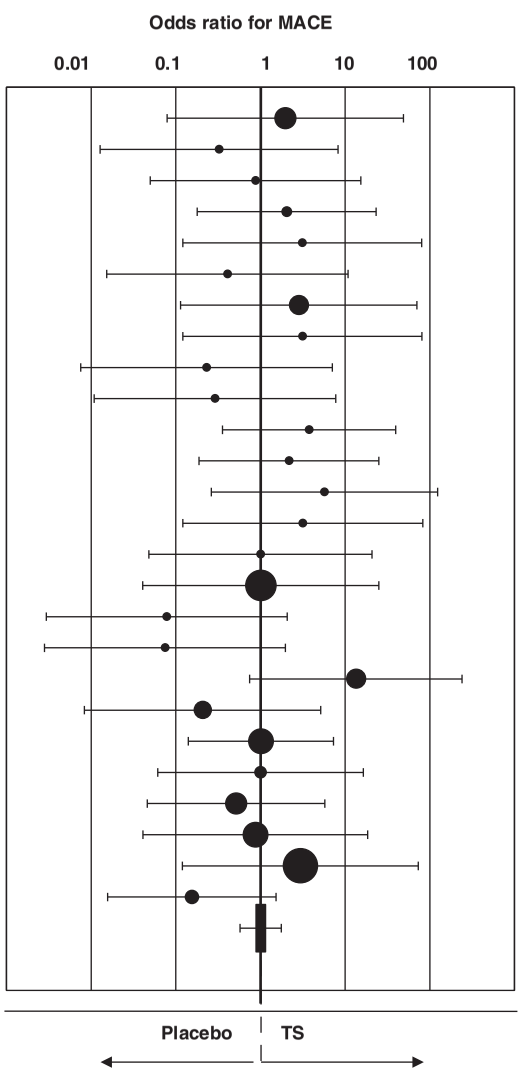
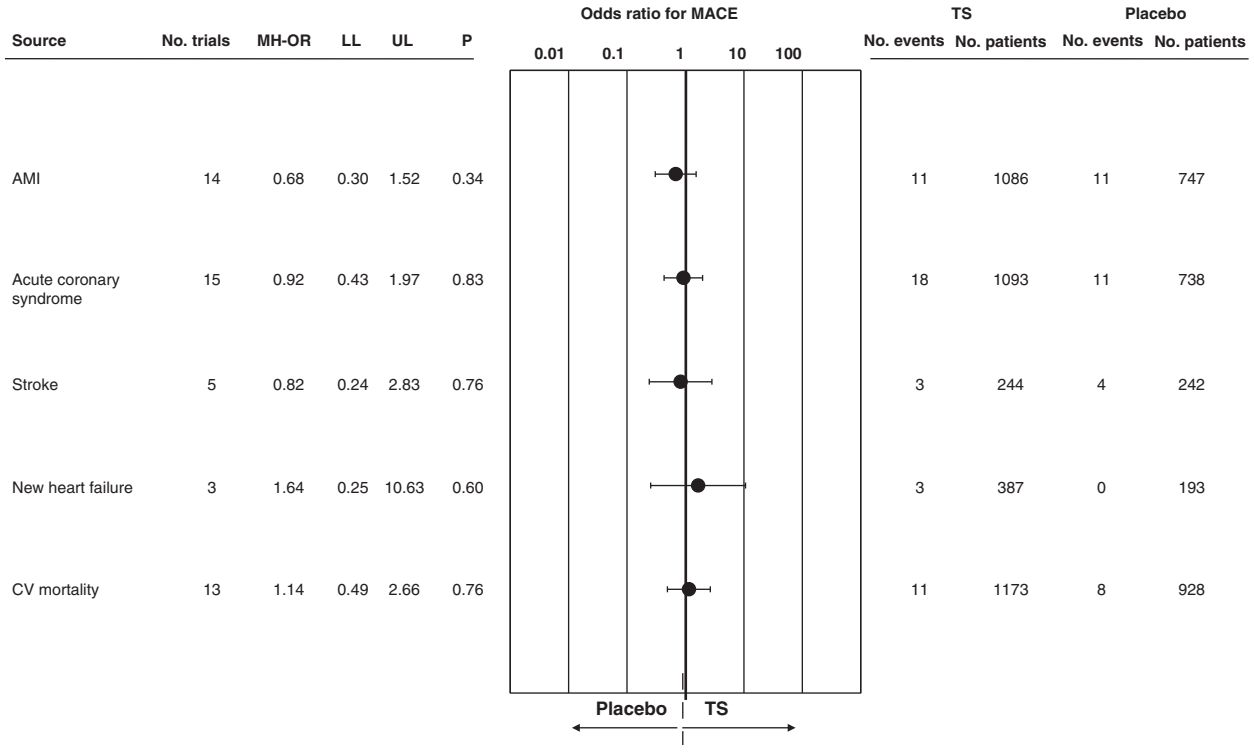


Figure 2. Odds ratio for major adverse cardiovascular events (MACE) in subjects treated with testosterone substitution (TS) or placebo. Among MACE, the authors considered cardiovascular death, non-fatal myocardial infarction and stroke, and acute coronary syndromes and/or heart failure.

LL: Lower limit; MH-OR: Mantel–Haenszel odds ratio; UL: Upper limit.



Source	No. trials	MH-OR	LL	UL	P	TS No. events	TS No. patients	Placebo No. events	Placebo No. patients
AMI	14	0.68	0.30	1.52	0.34	11	1086	11	747
Acute coronary syndrome	15	0.92	0.43	1.97	0.83	18	1093	11	738
Stroke	5	0.82	0.24	2.83	0.76	3	244	4	242
New heart failure	3	1.64	0.25	10.63	0.60	3	387	0	193
CV mortality	13	1.14	0.49	2.66	0.76	11	1173	8	928

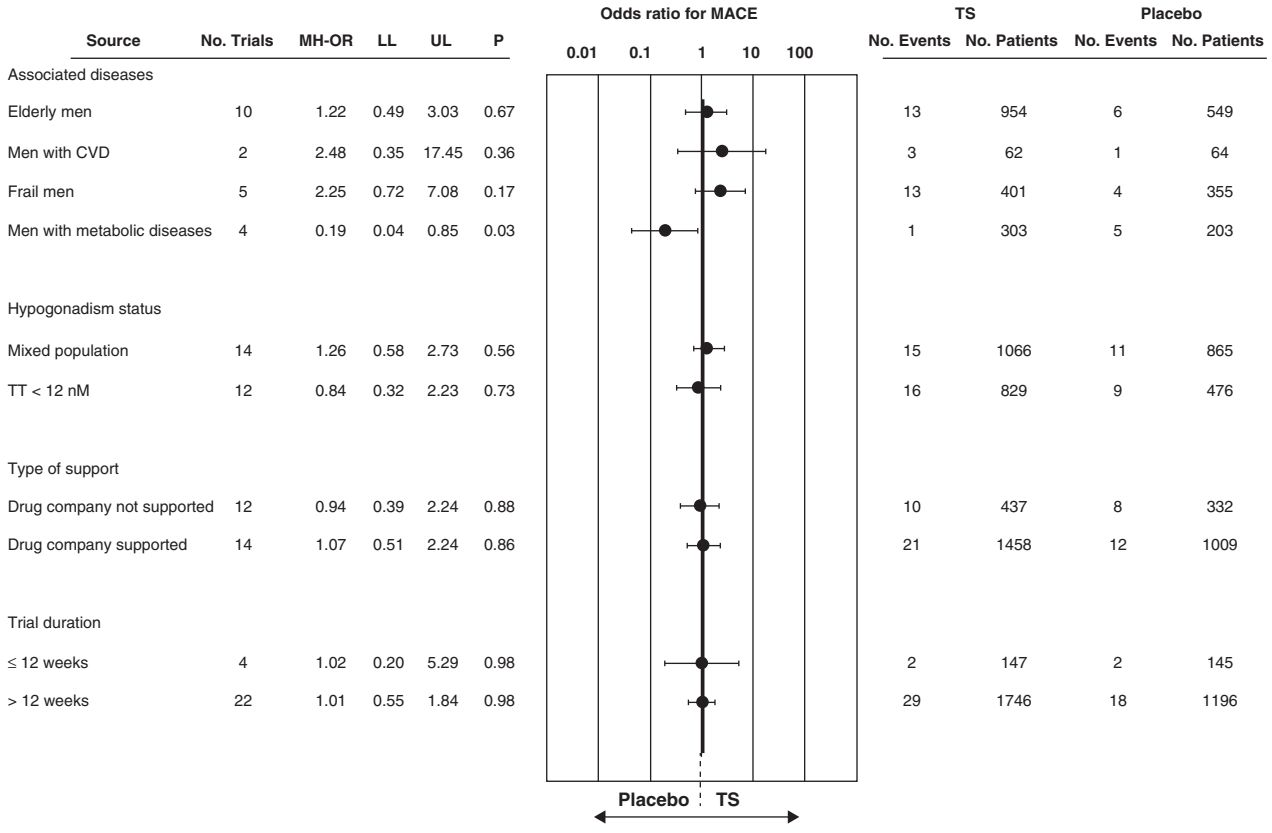
Figure 3. Odds ratio for acute myocardial infarction (AMI), acute coronary syndrome, stoke, heart failure and cardiovascular (CV) mortality in subjects treated with testosterone substitution (TS) or placebo.

LL: Lower limit; MACE: Major adverse cardiovascular events; MH-OR: Mantel–Haenszel odds ratio; UL: Upper limit.

7. Pharmaco-epidemiological and retrospective interventional studies

A retrospective observational study from Seattle evaluated mortality rate in a series of 1031 T-treated, compared with untreated hypogonadal (total T < 8.7 nmol/l) male veterans (VA) greater than 40 years old [18]. Over a mean follow-up period of 40.5 months, it was found that men receiving TS (n = 398) had a 39% decrease in mortality, when compared to the untreated counterpart [18]. Similar results have been reported in another retrospective study on type 2 diabetic subjects [19]. Over a mean follow-up of 5.8 years, those men with a low T (< 10.4 nmol/l) had a twofold increased risk of death as compared to the rest of the diabetic population. Accordingly, by meta-analyzing these studies, the authors found that the lack of TS in hypogonadal subjects doubled the risk of mortality [138]. However, these results should be interpreted cautiously because residual confounding may still be a source of bias, including the substantial risk of a primary selection bias due to the nonrandom assignment of T exposure. The mortality-related benefits of TS reported in these two retrospective interventional studies [18,19] could be related to a selection bias: physicians often prefer to treat healthier individuals, and healthier individuals more often request treatment for their hypogonadism-related (sexual) problems, accounting for mortality being lowest in this group.

In apparent contrast with previous findings are two recently published pharmaco-epidemiological studies. The first was published in the Journal of the American Medical Association (JAMA) last year [12]. It retrospectively evaluated a cohort of 8709 VA patients, who had undergone coronary angiography between 2005 and 2011 showing low T levels (T < 10.4 nmol/l). Some of the men received TS, while others did not. Among men who were receiving any form of TS, 25.7% had MACE, or died from any cause, versus 19.9% of those who did not receive hormonal therapy, with a hazard ratio of 1.29 (95% CI 1.04 – 1.58; p = 0.02), which was not substantially affected by adjusting for confounders [12]. Although the study presented many flaws, due to its retrospective design and to the little information on the VA database, it has received much attention in the lay media and prompted FDA to reassess the CV safety of T therapy [14]. Another retrospective study, funded by the National Institutes of Health, investigated, in a large healthcare database from Truven Health Analytics, the rate of nonfatal myocardial infarction in 56,000 middle-aged and older men, who were prescribed TS [13]. The authors compared the rate of heart events in the 90 days after starting TS with the rate in the prior year. The study reported a doubling in the risk of heart attack among men aged 65 years and older and a two- to threefold increased risk in younger men with a pre-existing history of heart disease, but not in those without CV events. For



Source	No. Trials	MH-OR	LL	UL	P	TS No. Events	TS No. Patients	Placebo No. Events	Placebo No. Patients
Associated diseases									
Elderly men	10	1.22	0.49	3.03	0.67	13	954	6	549
Men with CVD	2	2.48	0.35	17.45	0.36	3	62	1	64
Frail men	5	2.25	0.72	7.08	0.17	13	401	4	355
Men with metabolic diseases	4	0.19	0.04	0.85	0.03	1	303	5	203
Hypogonadism status									
Mixed population	14	1.26	0.58	2.73	0.56	15	1066	11	865
TT < 12 nM	12	0.84	0.32	2.23	0.73	16	829	9	476
Type of support									
Drug company not supported	12	0.94	0.39	2.24	0.88	10	437	8	332
Drug company supported	14	1.07	0.51	2.24	0.86	21	1458	12	1009
Trial duration									
≤ 12 weeks	4	1.02	0.20	5.29	0.98	2	147	2	145
> 12 weeks	22	1.01	0.55	1.84	0.98	29	1746	18	1196

Figure 4. Odds ratio for acute major adverse cardiovascular events (MACE) according to baseline characteristics in subjects treated with testosterone substitution (TS) or placebo. Among MACE, the authors considered cardiovascular death, non-fatal myocardial infarction and stroke, and acute coronary syndromes and/or heart failure.
CVD: Cardiovascular diseases; LL: Lower limit; MH-OR: Mantel-Haenszel odds ratio; TT: Total testosterone; UL: Upper limit.

comparison, the authors analyzed 167,000 individuals who were prescribed a PDE5i under the same conditions, and no increase in CV events was observed. When they followed-up men under TS for another 90 days, the risk declined to the level it was at the study's entry for men who did not refill their initial prescription. The analysis offers the advantage of using more stringent criteria for CV events – compared to the vague composite groups used in the previous study [13] and correlated the novel incident events early on with the early phase of TS. Although the authors found an association between T therapy and increased risk of heart attack, this study [13], as well as the previous one [12], cannot prove a cause- and effect-relationship. In addition, information obtained from medical records makes it uncertain which men actually used the T substitution. The comparison of TS with PDE5i is questionable, because the latter have distinct cardio-protective effects [9,124-127]. Finally, concerns about a small increase in risk as derived from retrospective evaluation of selected cohorts have been raised [139]. Nonetheless, the FDA on the basis of these two studies [12,13] urged "healthcare professionals and patients to report side effects involving prescription testosterone products to the FDA MedWatch program".

8. Conclusion

Testosterone may exert multiple actions on blood vessels and the heart ([140], see for review [141]). The pathophysiology underlying potential associations between testosterone and CV system is rather complex as the hormone has differing effects according to its binding to different receptors (androgen, estrogen, membrane-bound and cytosolic receptors) and interindividual variability. The best documented effect of TS is the stimulation of red blood cell production through the hepatic suppression of hepcidin, augmentation of erythropoietin, and a direct effect on bone marrow, including on erythroid progenitors, ferrokinetics and red cell precursor survival [140]. Hence, it is important to monitor hematocrit at regular intervals, to avoid potentially serious adverse events. In addition, pre-existing erythrocytosis constitutes a risk factor for thrombosis in hypogonadal men.

A hypogonadal status is often associated with CVD, representing for clinicians a warning of poor general health and a possible biomarker of disease severity. This might justify the increased mortality associated to T deficiency [20-22]. The observation of an epidemiological association between a

Source	MH-OR	LL	UL	P	TS No.Events	TS No. Patients	Placebo No.Events	Placebo No. Patients
Copenhagen SG 1986 [31]	2.22	0.78	6.31	0.13	16	134	5	87
Hall *et al.*, 1996 [34]	0.19	0.01	4.08	0.29	0	35	2	35
Sih *et al.*,1997 [36]	0.88	0.05	15.33	0.93	1	17	1	15
Snyder *et al.*, 1999 [40]	1.96	0.61	6.29	0.26	9	54	5	54
English *et al.*, 2000 [42]	5.43	0.25	118.96	0.28	2	25	0	25
Seidman *et al.*, 2001 [47]	0.41	0.02	10.83	0.59	0	13	1	17
Steidle *et al.*, 2003 [52]	4.76	0.23	100.40	0.32	2	106	0	99
Amory *et al.*, 2004 [54]	5.44	0.25	119.63	0.28	2	24	0	24
Kenny *et al.*, 2004 [56]	0.23	0.01	7.05	0.40	0	6	1	5
Svartberg *et al.*, 2004 [60]	0.29	0.01	7.74	0.46	0	15	1	14
Brockenbrough *et al.*, 2006 [62]	1.20	0.34	4.18	0.77	9	19	9	21
Malkin *et al.*, 2006 [69]	0.86	0.24	3.10	0.82	5	37	6	39
Merza *et al.*,2006 [71]	0.30	0.01	7.85	0.47	0	20	1	19
Nair *et al.*, 2006 [72]	1.32	0.39	4.50	0.66	7	30	6	32
Okun *et al.*, 2006 [73]	0.46	0.04	5.75	0.55	1	15	2	15
Emmelot-Vonk *et al.*, 2008 [72]	2.35	0.59	9.33	0.22	7	120	3	117
Svartberg *et al.*, 2008 [81]	3.16	0.12	82.64	0.49	1	19	0	19
Caminiti *et al.*, 2009 [82]	2.06	0.18	23.83	0.56	2	35	1	35
Chapman *et al.*, 2009 [84]	1.00	0.05	20.83	1.00	1	6	0	6
Legros *et al.*, 2009 [85]	1.01	0.04	25.01	1.00	1	237	0	79
Aversa *et al.*, 2010 [88]	0.08	0.00	2.07	0.13	0	40	1	10
Aversa *et al.*, 2010 [90]	0.07	0.00	1.97	0.12	0	42	1	10
Basaria *et al.*, 2010 [11]	6.05	2.22	16.51	0.00	25	106	5	103
Kalinchenko *et al.*, 2010 [91]	0.12	0.01	2.59	0.18	0	113	2	71
Srinivas-Shankar *et al.*, 2010 [93]	2.60	0.49	13.61	0.26	5	136	2	138
Ho *et al.*, 2011 [95]	1.00	0.14	7.34	1.00	2	60	2	60
Jones *et al.*,2011 [96]	0.40	0.14	1.19	0.10	5	108	12	112
Kaufman *et al.*, 2011 [97]	1.49	0.33	6.71	0.60	17	234	2	40
Hoyos *et al.*, 2012 [98]	3.18	0.13	81,01	0.48	1	33	0	34
Hildreth *et al.*, 2013 [101]	0.14	0.04	0.48	0.00	4	96	11	47
NCT00957528	0.88	0.05	16.74	0.93	1	9	1	8
Overall	**1.07**	**0.69**	**1.65**	**0,76**	**126**	**1944**	**83**	**1390**

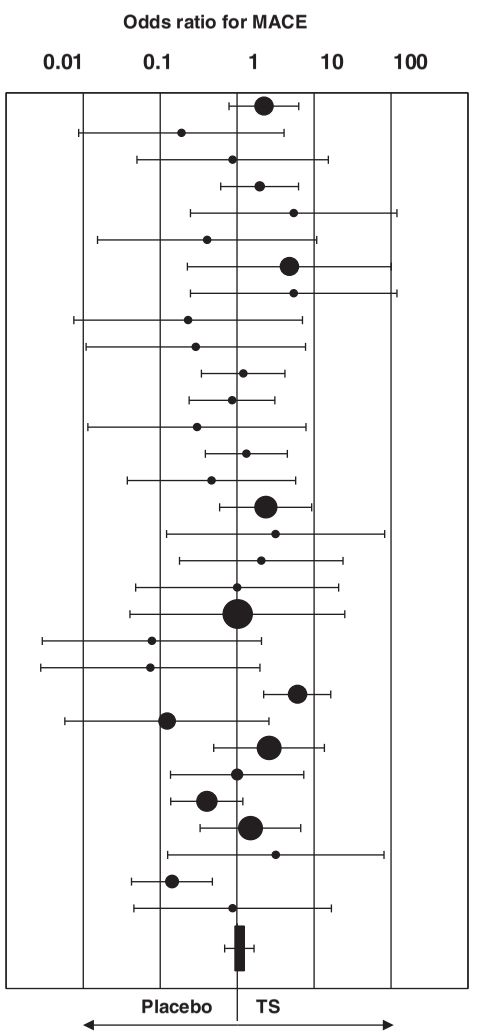


Figure 5. Odds ratio for overall cardiovascular (CV) events in subjects treated with testosterone substitution (TS) or placebo.

LL: Lower limit; MH-OR: Mantel-Haenszel odds ratio; UL: Upper limit.

clinical condition (low T) and CV- and overall-mortality might prompt clinicians to treat the supposed risk factor aggressively. However, careful consideration of the mechanisms involved is always necessary prior to translating observational data into therapeutic decisions. In fact, many epidemiological associations are determined by adaptive mechanisms and not by pathogenetic factors; the treatment of a compensatory alteration can easily produce a detrimental effect. Retrospective interventional studies have provided conflicting results: two indicated that TS improves survival of treated hypogonadal men [18,19] and two the opposite effect, with an increase in unfavorable CV events [12,13]. The present systematic review of the available evidence and meta-analysis of RCTs did not substantiate the view of T treatment as carrying any additional risk of CV-related adverse events, when hypogonadism is properly diagnosed and replacement therapy correctly performed. Finally, the authors observed a possible protective role of TS in subjects with metabolic disease.

Based on hypothetical aphrodisiac and anabolic effects, many middle-aged and older men beg their doctors for TS. The authors now clearly state that the positive effect of TS in otherwise eugonadal men has not yet been documented. In addition, it is important to note that in many countries, including ours, TS is approved only for subjects with demonstrated hormone deficiency and associated symptomatology. Hence, TS must not be undertaken lightly and prescribed without an appropriate endocrinological testing and diagnosis. In addition, TS should be properly monitored by a specialist that is well versed in the risks of therapy and the treatment of possible side effects to have optimal benefits from the therapy. On the other hand, based on recent media warnings about the risk of heart attacks and other CV problems associated with TS, hypogonadal patients should not stop taking prescribed T products without first discussing any questions or concerns with their healthcare professionals.

9. Expert opinion

The recently published pharmaco-epidemiological studies [12,13], along with one RCT (with supra-physiological doses of TS [11] and one meta-analysis ([28]; clearly discordant with all the previous ones and with the present meta-analysis) have induced an epidemic of sensational, often misleading, media coverage and false claims on the potential dangerous effect of TS on the CV system. Every other day popular press is terrifying their audience with announcements of serious T-associated cardiac hazard, only based on clinical epidemiology observation. The authors should recognize that pharmaco-epidemiology has led to important discoveries, such as the positive effect of aspirin on myocardial infarction [142]. However, observational, clinical research suffers from important biases (including selection, information, confounding biases) and caution is needed when evaluating results reporting small differences [139]. This is the case for the two aforementioned observational studies [12,13], where differences between raw event numbers in patients not receiving treatment versus those in the active arm were small and hazard ratio well below thresholds of high confidence. Juggling the risk factors and hazard ratio – in particular by using large registry databases – could transform coincidence into causality, with important inferences in everyday life and public health, for example, forcing hypogonadal men to abandon their therapy for hypothetical reasons.

Properly powered placebo-RCTs with a primary CV end point, specifically designed for middle-aged and older hypogonadal men are still lacking, although anxiously awaited by scientists in the field. Systematic reviews and meta-analyses are often considered as the highest level of evidence for evaluating interventions in healthcare and as a particularly useful tool to address questions for which multiple data sources are conflicting, or when there is a variety of reports with low statistical power, because pooling data can improve power and provide a convincing result. Therefore, meta-analytic surveys collecting the occurrence in CV adverse events in men treated with T or placebo in RCTs otherwise designed are welcome. However, methodological issues including inconsistencies in the reporting of adverse events in trials without prior formal adjudications, along with the limited reliability of diagnostic criteria for diagnosing such events, limit their final interpretation. In fact, the meta-analysis of CV events in trials with non-CV primary end points has several limitations, most notably the lack of predefined diagnostic criteria and screening methods for incident CVD, with the risk of misdiagnosis and under-diagnosis. In addition, the duration of available RCTs is rather short, not allowing inferences on longer term effects. Finally, combining data of flawed evidence does not remove biases. This is the main reason why the authors primarily focused on MACE, a more robust diagnostic category, which are easier to detect and less controversial in diagnosis.

Present systematic analysis of available evidence and our expert opinion does not support a causal role between TS and adverse CV events when hypogonadism is properly diagnosed and replacement therapy correctly performed. Our view is in agreement with a large body of literature from the last 20 years supporting TS as an important strategy in improving insulin sensitivity and glucose levels, reducing body fat and increasing lean muscle mass, all factors that would reduce the risk of heart disease [143]. Accordingly, by meta-analyzing studies involving subjects with T2DM or MetS, the authors even identified a protective effect of TS against MACE [3,136,137]. However, the number of studies included was too small to draw final conclusions. Whereas interventions against the comorbidity (such as MetS and T2DM) underlying hypogonadism are obviously advisable, those aimed at restoring slightly decreased hormonal levels are more questionable, in the case that the latter condition represents a potentially compensatory mechanism. Results from targeted randomized-controlled trials are more than

welcome to delineate the risk and benefits of TS in subjects with CVD.

Declaration of interest

G Corona has received consultancy fees from Bayer, Besins, Otsuka, Eli-Lilly and Menarini, M Maggi has received consultancies from Bayer, Prostrakan, GSK, Eli-Lilly and Menarini and AM Isidori has received consultancies from Bayer, Besins, Otsuka, and Menarini. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Affiliation

Giovanni Corona[1], Elisa Maseroli[2], Giulia Rastrelli[2], Andrea M Isidori[3], Alessandra Sforza[1], Edoardo Mannucci[4] & Mario Maggi[†2]

[†]Author for correspondence

[1]Azienda-Usl Bologna, Maggiore-Bellaria Hospital, Medical Department, Endocrinology Unit, Bologna, Italy

[2]University of Florence, Department of Experimental, Clinical and Biomedical Sciences, Sexual Medicine and Andrology Unit, Viale Pieraccini 6, 50139 Florence, Italy

Tel: +39 55 4271415;

Fax: +39 55 4271413;

E-mail: m.maggi@dfc.unifi.it

[3]Sapienza University of Rome, Department of Experimental Medicine, Rome, Italy

[4]Careggi Hospital, Diabetes Agency, Florence, Italy

Supplementary materials available online

Supplementary file 1.