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Cardiovascular safety of testosterone replacement therapy in men: an updated systematic review and meta-analysis

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Abstract

Introduction: The cardiovascular (CV) safety of testosterone (T) replacement therapy (TRT) is still conflicting. Recent data suggested a TRT-related increased risk of atrial fibrillation (AF). To systematic review and meta-analyze CV risk related to TRT as derived from placebo controlled randomized trials (RCTs).

Areas covered: An extensive Medline, Embase and Cochrane search was performed. All placebo-controlled RCTs reporting data on TRT-related CV safety were considered. To better analyze the role of T on AF, population-based studies investigating the relationship between endogenous circulating T levels and AF incidence were also included and analyzed.

Expert opinion: Out of 3.615, 106 studies were considered, including 8.126 subjects treated with TRT and 7.310 patients allocated to placebo. No difference between TRT and placebo were observed when major adverse CV events were considered. Whereas the incidence of non-fatal arrhythmias and AF was increased in the only trial considering CV safety as the primary endpoint, this was not confirmed when all other studies were considered (MH-OR 1.61[0.84;3.08] and 1.44[0.46;4.46]). Similarly no relationship between endogenous T levels and AF incidence was observed after the adjustment for confounders Available data confirm that TRT is safe and it is not related to an increased CV risk.

Key words: Atrial fibrillation, non-fatal arrhythmias, MACE, hypogonadism, testosterone, testosterone replacement therapy.

Article highlights:

- Data from TRAVERSE study suggested an increased risk of non-fatal arrhythmias and atrial fibrillation in subjects treated with testosterone in comparison with placebo.
- Data derived from randomized placebo controlled trials with cardiovascular safety as secondary endpoint (non TRAVERSE studies) do not confirm the increased risk of non-fatal arrhythmias and atrial fibrillation in relation of testosterone replacement therapy.
- TRAVERSE and non-TRAVERSE studies showed a neutral effect of testosterone replacement therapy when major adverse cardiovascular events were considered .
- Data derived from population-based studies investigating relationships between endogenous T levels and non-fatal arrhythmias suggest a possible protective role for T on AF, although the association was not confirmed in a fully adjusted model or when outliers' studies were excluded.

1.0 Introduction

During the last three decades, there has been an increasing awareness concerning age-associated testosterone (T) deficiency and its potential treatment. Although the phenomenon of an age-associated T decline was supported by several epidemiological studies [1-4], its clinical relevance is still unclear and a matter of intense debate. In fact, it is not clear whether the reduced T levels observed in the aging male contribute to the age-related morbidities and symptoms or whether low T and associated morbidities are concomitant conditions, both associated with the aging process [5]. Nonetheless, the pharmaceutical sales of T-containing medications dramatically increased worldwide with a 100-fold rise in thirty years [6]. This trend was clearly apparent in the North American market. In fact, the defined monthly doses per 1000 population per year of T transdermal preparation rose over 11 years from 10 and 10.3 to 385 and 98.5 in Canada and the US, respectively [6]. This figure was also apparent in Northern Europe with a raise from 4.5 to 22.1, but less in Southern Europe, where the increasing trend over 11 years was relatively modest (from 5.6 to 7.2). In North America, the major factors responsible for this phenomenon were not an increased prevalence of T deficiency - which indeed is very similar worldwide - but direct-to-consumer advertising (DTCPA), the presence of Internet pharmacies and intense disease mongering [6]. In fact, T treatment was often promoted as a fountain of youth [5,6]. A survey, conducted in several American market areas, has demonstrated that between 2009 and 2013 exposure to televised DTCPA was associated with greater T testing, new initiation of therapy and, especially, initiation of therapy without prior T testing [7].

To partially limit this T overuse, the US Food and Drug Administration (FDA) cautioned that the benefits and safety of treatment with T products have not been clearly established for the treatment of low T levels due to aging [8,9]. In particular, the FDA stated that testosterone replacement therapy (TRT) should be considered only for men with “classical hypogonadism”, i.e. due to primary or secondary T deficiency resulting from known problems within the testis, pituitary, or

hypothalamus [8]. Later on, the concept of non-classical hypogonadism (HG) or *functional* HG was partially incorporated in the clinical practice guidelines of the US Endocrine Society [10] as well as in the Australian one [11], as opposed to *organic* (classic) HG. In addition, the FDA issued a safety notification regarding the misuse of T-containing products due to a potential risk of cardiovascular (CV) harm [8]. In particular, FDA recommended against the use of T supplementation in men with a hormone deficiency not due to organic causes but age or comorbidities, i.e. functional HG [8]. In fact, Grossmann & Matsumoto, in a perspective article [12], considered the risks of CV diseases (CVD) as "unknown" in functional HG, while they were "low relative to benefits" in the organic one. This statement is not evidence-based because we are not aware of any trials enrolling subjects with organic HG and facing the risk over the benefit of TRT. Conversely, the majority of available trials with TRT were enrolling subjects with functional HG, such as the recently published Testosterone Treatment in Older Men trials (TTrials; [13]), the Testosterone treatment to prevent or revert type 2 diabetes in men enrolled in a lifestyle program trial (T4DM;[14]), and the Testosterone Replacement Therapy on the Incidence of Major Adverse Cardiovascular Events (MACE) and Efficacy Measures in Hypogonadal Men trial (TRAVERSE; [15]). In the real world, the large majority (85%) of subjects visiting an outpatient clinic for sexual dysfunctions, which represent the most specific complex of symptoms associated with adult-onset HG, are classified as functional HG, as demonstrated by an analysis of more than 4000 individuals [16].

The FDA's position on possible CV risk associated with TRT was essentially based on three studies published almost 10 years ago [17-19]. Two of them were pharmaco-epidemiological studies [18,19] and one was prematurely interrupted randomized placebo-controlled trial not powered to detect differences in CV and not having CV events as a primary end-point [17]. Specific criticisms of these three studies are reported elsewhere [16]. Of note, the European Medicine Agency (EMA), after conducting a similar review of the data as the FDA, did not find sufficient evidence for declaring TRT to be associated with an increased CV risk [20]. The large majority of the meta-analyses conducted so far on randomized placebo-controlled trials (RCT) failed to demonstrate an

increase in CV risk in subjects with functional HG receiving TRT [21-29], including a recent individual patient and aggregate meta-analysis [30]. However, studies included in the aforementioned meta-analyses do not have CV events as primary end-points, used a broad definition of them and CV events were often not adjudicated.

Until recently, only few RCTs were available with subjects with CV outcomes as the primary end-point in patients with coronary heart disease (CHD); the meta-analysis of these trials did not provide any signal for an increase in CV risk associated with T administration [31]. In fact, in the six RCTs meta-analyzed, enrolling 258 patients with CHD with a mean follow-up of 23 weeks, TRT was positively associated with a significant increase in treadmill test duration and time to 1 mm ST segment depression[31]. However, the small number of patients and the short follow-up strongly limited the interpretation of the results.

Considering the intense debate around the safety of TRT, in March 2015 the FDA released a requirement that manufacturers of FDA-approved T therapies conduct prospective studies of sufficient size and duration to evaluate whether their products are associated with an elevated risk of CV events. Hence, the TRAVERSE study was thereafter prompted [15]. The TRAVERSE study recruited 5246 men aged 45-80, predominantly with pre-existing or high-risk CV disease and a total T<10.4 nmol/L with self-reported sexual dysfunction or other symptoms characteristic of functional HG. Men with congenital or acquired severe organic HG were not included in the study. Patients were randomly allocated to receive transdermal T gel (1.6%) or placebo for an average of 21.7±14.1 months and after 33 months of post-treatment follow-up, with a discontinuation rate of 61% in both groups. The primary CV end-point was the first occurrence of any CV events of a composite of cardiovascular death, nonfatal myocardial infarction, and nonfatal stroke. The secondary outcome was the first recurrence of the composite outcome plus coronary revascularization. The first adjudicated major adverse CV event (MACE) occurred in 182 patients (7.0%) in the T group and in 190 patients (7.3%) in the placebo group. No apparent clinically meaningful differences in the

incidence of secondary CV end-point events were observed between the trial groups. Hence, no increase in MACE risk was detected in this large study [15]. However, in the T arm of the TRAVERSE trial nonfatal arrhythmias occurred in 134 patients (5.2%) and atrial fibrillation (AF) in 91 patients (3.5%), while the same events in the placebo group occurred in 87 (3.3%) and 63 patients (2.4%), respectively, reaching statistical significance [15].

The aim of this study is to compare results obtained in the TRAVERSE trial on CV risk [15] with those available in other trials, with MACE not as a primary end-point, by using a meta-analytic approach. In addition, it will also be investigated whether, as revealed by the TRASVERSE trial [15], there is a potential negative effect of TRT on nonfatal cardiac arrhythmias by using data from other available trials. A possible association between endogenous T and atrial fibrillation will also be explored in available epidemiological studies.

2.0 Methods

This meta-analysis was performed in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (see Supplementary File 1). The protocol of this study (CRD42023474558) was published on the website of the University of York (Centre for Reviews and Dissemination,https://www.crd.york.ac.uk/PROSPERO/#recordDetails).

2.1 Search strategy

An extensive Medline, Embase and Cochrane search was performed, including the following MeSH terms (‘testosterone’AND/OR clinical trials) for the selection of all placebo-controlled RCTs for the analysis of the same endpoints. The search was limited to those paper related to “humans” and published in “English” language.

In addition, a second separate search was performed, including the following MeSH terms (testosterone AND/OR atrial fibrillation).

The search, which accrued data from January 1, 1969 up to September 30th, 2023, was restricted to English-language articles and studies of human participants. The identification of relevant studies was performed independently by three of the authors (G.R., C.S., G.C.), and conflicts were resolved by the third investigator (M.M.). We did not employ search software. We hand-searched the bibliographies of retrieved papers for additional references. The principal source of information was derived from published articles. If data were missing from a publication, an attempt at retrieval was made through the clinicaltrials.gov website.

2.2 Study selection

We included all placebo-controlled RCTs evaluating the effects of TRT versus placebo on different endpoints. All studies without any arbitrary restriction, even if CV events were not the principal endpoints, were included ([14,15,17,32-133]; see also Supplementary Figure 1, Panel A and Table 1, and Supplementary Tables 1-2). Studies not specifically stating the occurrence or absence of CV-related events were excluded from the analysis. Studies using androgens other than T, 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. Finally, since phosphodiesterase type 5 inhibitors (PDE5is) have been reported to have a possible positive influence on CV outcomes, RCTs evaluating the effect of TRT as an add-on to PDE5i were excluded from the analysis.

During the second search, all population-based studies investigating the relationship between T circulating levels and the incidence of AF were analyzed [134-138]. Data derived from populations different from dwelling subjects were excluded from the analysis. Similarly, studies reporting only

data on arrhythmias different from AF were also not considered (see also Supplementary Figure 1, Panel B, and Table 2, and Supplementary Table 3).

2.3 Outcome

The principal outcome of this analysis was to evaluate the effect of TRT compared to placebo, on the incidence of new MACE. MACE was defined as the composite of CV death, non-fatal acute myocardial infarction (AMI) and stroke, and acute coronary syndromes and/or heart failure (HF) reported as serious adverse events. Secondary outcomes included the incidence of overall non-fatal arrhythmias and AF.

2.4 Quality assessment

The quality of RCTs was assessed using the Cochrane suggestions [139] (see Supplementary Table 2). The quality assessment of the population based studies was evaluated according to Newcastle Ottawa tool indications [140] (Supplementary Table 3). In particular, in RCTs, the following risks of bias were evaluated [139]: arising from the randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported results. For each interventional study, we also assessed how the population was selected, the duration and route of TRT the adequacy of study follow-up, and the funding source. In addition, according to Newcastle Ottawa tool indications [140], each study is judged on 8 items, categorized into 3 groups: the selection of the study groups; the comparability of the groups; and the ascertainment of the outcome of interest.

2.5 Statistical analysis

Heterogeneity was assessed by using Chocran's Q and I^2 statistics. Even when 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 we used funnel plots and the Begg-adjusted rank correlation test [141]. However, because these tests have low statistical power when the number of trials is small, undetected bias may still be present. In RCTs a Mantel-Haenszel odds ratio with a 95% Confidence Interval (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, a sub-analysis considering the incidence of MACE according to baseline population characteristics was also performed. For the second analysis MH-OR with 95% was calculated for the incidence of non-fatal arrhythmias and AF in relation to baseline T levels.

All analyses were performed using Comprehensive Meta-analysis Version 2, Biostat (Englewood, NJ, USA).

3.0 Results

3.1 Descriptive statistics

Out of 3.615, 106 studies [14,15,17,32-133] were included, accounting for 8.126 subjects treated with TRT and 7.310 patients allocated to placebo, respectively. In particular, the TRAVERSE study included 2.601 and 2.603 patients in the active and comparator arms, respectively. All other non-TRAVERSE trials enrolled 5.525 and 4.707 subjects treated with T and placebo, respectively. The mean follow-up of the people involved in the TRAVERSE Study was 86.8 weeks whereas other studies were characterized by a shorter mean duration of 33.6 weeks. The mean age between subjects included in the TRAVERSE study and those patients enrolled in the other trials was similar (63.3 vs. 60.0 years, respectively). Conversely, the TRAVERSE study included individuals with a

higher body mass index (BMI) and lower T levels (34.9 vs. 28.8 kg/m^2 and 7.8 vs. 11.0 nmol/L, respectively). Data on MACE as well as on arrhythmias and atrial fibrillation were all specified in the TRAVERSE study. When overall data derived from other studies were considered, information on MACE, acute myocardial infarction (AMI), stroke, and CV mortality was reported in 104, 101, 102 and 105 respectively. In addition, 100 studies also reported information on acute coronary syndrome, and 102 on hospitalization for HF. Finally, data on arrhythmias and atrial fibrillation (AF) were reported by 99 and 91 respectively. The characteristics of the retrieved trials (including parameters on trial quality) and the number of events recorded are reported in Table 1 and Supplementary Tables 1 and 2.

The TRAVERSE study included only hypogonadal subjects (total T < 10.4 nmol/L), treated with daily transdermal 1.62% T gel, and adjusted to maintain T levels between 12 and 25.7 nmol/L. Conversely, other considered non-TRAVERSE trials differed in the characteristics of the enrolled patients and in the type and dosage of T preparations used (Table 1). In particular, 61 RCTs evaluated the effect of TRT in a mixed population of hypogonadal and eugonadal subjects and 45 in hypogonadal patients (T below 12 nmol/L). In addition, eight trials used oral T formulations, whereas 53 and 44 studies employed intramuscular and transdermal T preparation. Finally, in one RCT, both intramuscular and transdermal T preparations were used.

3.2 MACE-related TRT- risk

Out of 104 trials reporting information on MACE, 36 studies detected at least one event, whereas in 68 studies no event was reported. Hence, the main analysis was performed on 36 trials. Q and I^2 were 35.0 and 0.0, respectively (p=0.69). The funnel plot and Begg adjusted rank correlation test (Kendall's τ: −0.14; p=0.22) suggested no major publication bias. When overall MACE were considered, no difference in TRT-related risk was observed when data derived from the

TRAVERSE study were compared to the rest of the studies (MH-OR: 1.05[0.90; 1.28]; p=0.52 vs. 0.89[0.63; 1.28]; p=0.54) (Figure 1 and Supplementary Figure 2). A sensitivity analysis was performed with continuity correction, confirming the results of the main analysis when overall MACE in nonspecific populations were considered (MH-OR: 0.91[0.73; 1.14]; p=0.42). Similarly, no difference in the individual MACE between data derived from the TRAVERSE study or other trials was also observed (Figure 1).

In addition, no risk in the population of non-TRAVERSE studies was detected when the heterogeneity was decreased (Q < 20%; Q=18.98), excluding from the general meta-analysis the outlier studies, defined as the ones with estimates beyond the overall measured effect [94,95] or with a very wide 95% CI [17,114] (MH-OR=0.80[0.54;1.17]; p=0.25).

Similarly, no risk between TRT and placebo was detected when specific characteristics including age, duration of follow-up, reported industry support, baseline T levels, and type of T preparation used (Supplementary Figure 3). However, when studies using T dosages above the suggested recommendations were considered, a higher risk of MACE was detected (MH-OR= 2.79[1.18;6,63]; p=0.02; see also Supplementary Figure 3). In addition, a protective role was observed when trials enrolling obese (BMI> 30 kg/m^2) patients were considered (MH-OR= 0.59[0.37;0.95]; p=0.03; Supplementary Figure 3). However, the latter observation was not confirmed when the TRAVERSE study (mean BMI 34 kg/m^{2}was included in the analysis (MH-OR= 0.85[0.63;1.15]; p=0.30).

3.3.0 Non fatal-arrhythmias and atrial fibrillation

3.3.1 Non fatal -arrhythmias and atrial fibrillation TRT-related risk

Among the 99 studies reporting information on nonfatal arrhythmias, at least one event in TRT or placebo arms was reported in 15 studies, whereas 84 did not find any event (Supplementary Figure

2). Overall, 4.270 subjects in the active arm and 4.055 individuals in the placebo arm were included. Data from the TRAVERSE trial showed an increased risk of overall nonfatal arrhythmias when TRT was compared to placebo (Figure 2). When non-TRAVERSE studies were considered, Q and I^2 were 10.3 and 0.0, respectively (p=0.74). The funnel plot and Begg-adjusted rank correlation test (Kendall's τ: 0.0; p=1.0) suggested no major publication bias. Overall, a trend towards a higher risk in the TRT arm was observed (Figure 2; Supplementary Figure 4 A). However, the latter association was not confirmed when only those trials enrolling hypogonadal (total T < 12 nmol/L) subjects were considered or when studies dealing with frail patients were excluded from the analysis (Figure 2 and Supplementary Figure 4, Panels B and C). Similar results were detected when trials using a dosage of TRT higher than current recommendations were excluded from the analysis (Supplementary Figure 4 Panel D).

In line with to what was reported for overall non fatal-arrhythmias, a higher risk of AF in TRT arm was reported in the TRAVERSE study (Figure 2). Among other non-TRAVERSE trials, only five RCTs reported information on AF. The latter studies included 688 and 684 patients in the TRT and placebo arms, respectively. No difference in AF risk between TRT and placebo was observed, although a tendency was evident (Figure 2, Supplementary Figure 5, Panel A). Similar results were observed, including only patients with hypogonadism (total T < 12 nmol/L) or excluding frail subjects and those who were prescribed too high dosage of TRT (Supplementary Figure 5, Panels B and C).

3.3.2 Epidemiological data

To better understand the contribution of T to AF incidence, a further analysis was performed by investigating the possible relationship between T circulating levels and incident AF, as derived from population-based studies. Out of 53, five surveys provided information on the latter topic [134-138]. The retrieved studies included 185,852 subjects with a mean follow-up of 12,04 years. In addition, the mean age, baseline T, BMI, and prevalence of active smokers of the enrolled individuals were 59.4 years, 14.8 nmol/L, 27.3 kg/m^2, and 17.6%, respectively. The characteristics of the included studies are reported in Table 2. When fully adjusted data were considered, Q and I^2 were 32.67 and 81.6, respectively ($p< 0.001$). The funnel plot and Begg-adjusted rank correlation test (Kendall's τ: 0.10; $p=0.76$) indicated no major publication bias. The age-adjusted data documented that low endogenous T levels at baseline were associated with a higher risk of incident AF (Figure 3 and Supplementary Figure 6, Panel A). The latter association was not confirmed when fully adjusted data were considered (Figure 3 and Supplementary Figure 6, Panel B). Finally, when the same analysis was performed by excluding the outlier studies in order to reduce the heterogeneity (< 50%; Q=40.7) the association between low T and an increased risk of incident AF was not confirmed even in the fully adjusted model, although a trend was evident (Figure 3 and Supplementary Figure 6, Panel C).

4.0 Conclusions

Present data show that TRT has a neutral effect on MACE risk, either when TRAVERSE or other (non-TRAVERSE) trials are considered. Similar results were observed when individual CV events composing MACE were considered. In addition, among non-TRAVERSE studies, no difference in MACE risk was observed when several aspects, including subject age, duration of follow-up, reported industry support, baseline T levels, and type of T preparation used, were considered. Conversely, a higher MACE risk was detected in those studies reporting T dosages above the suggested recommendations. Although a lower risk of MACE was observed when trials enrolling

obese (BMI> 30 kg/m^2) patients were analyzed in non-TRAVERSE trials, the inclusion of the TRAVERSE results into the meta-analysis did not confirm the association.

The present meta-analysis of epidemiological studies investigating relationships between endogenous T levels and non-fatal arrhythmias suggests a possible protective role for T on AF, although the association was not confirmed in a fully adjusted model or when outliers' studies were excluded. In contrast, the TRAVERSE study (15) showed an increased, and not a decreased, risk of arrhythmias and, in particular, of AF. It is important to note that, in the TRAVERSE trial [15], cardiac arrhythmias (AF and non-fatal arrhythmias) were not trial endpoints but investigator-reported adverse events. When a similar analysis of investigator-reported arrhythmias was performed in other non-TRAVERSE trials, we found a non-significant trend towards an increased risk of TRT-related cardiac arrhythmias (including AF), which was further substantially smoothed when trials involving frail subjects were excluded.

5.0 Expert opinion

The TRAVERSE trial, a properly powered placebo-RCT with a primary CV endpoint specifically designed for middle-aged and older symptomatic men with functional hypogonadism [15], substantially confirms the results of the previous [21-29] and the present (updated) meta-analyses. The odds ratio for AMI, acute coronary syndrome, stroke, heart failure, MACE, and overall CV mortality were equally null for either the present meta-analytic results or the TRAVERSE trial. Although meta-analyses are often considered the highest level of evidence for evaluating interventions in healthcare, they suffer from several methodological issues. In fact, they summarize CV events in RCTs designed for other purposes, with possible inconsistencies in the reporting of adverse events often without prior formal adjudication, along with limited reliability of diagnostic criteria for diagnosing and interpreting CV events. Hence, it is important that the TRAVERSE trial,

having MACE as a primary endpoint, confirms present and previous [21-29]meta-analytic results. In fact, the present head-to-head comparison between the TRAVERSE and other meta-analyzed trials substantially halts the previous dubs on the relative CV safety of TRT in middle-aged or older men with symptomatic *functional* hypogonadism. It is important to note that the TRAVERSE trial enrolled only men with acquired *functional* HG, with those with congenital or severe acquired HG explicitly excluded. The large majority of the other trials included in the present meta-analysis also enrolled men with characteristics compatible with *functional* hypogonadism. The present meta-analysis supports an even more protective role for TRT against MACE in trials enrolling obese men, as previously reported [28] or suggested by other authors in observational studies [142,143]. Considering that TRT can improve body composition in hypogonadal men by reducing fat mass and improving lean mass [14,144-146], this can explain its protective effect on CV risk in obese individuals. However, the introduction of TRAVERSE data into the meta-analysis did not substantiate the negative association between obesity and CV risk, most probably because the enrolled subjects in the TRAVERSE study were already at high CV risk. It is important to note that all analyzed trials, including the TRAVERSE one, on TRT and CV risk are of relatively short duration (i.e., a mean of 34 and 87 weeks, respectively), not allowing the inferences on longer-term effects of TRT. However, the number of observations collected in the present analysis is relevant. In fact, it includes 2,601 and 2,603 patients in the active and comparator arms of the TRAVERSE trial, along with 5,525 and 4,707 subjects treated with T and placebo, respectively, in the other trials here meta-analyzed. Hence, in total, we here report 15,436 observations from the available RCT on TRT-related CV risk, suggesting the overall safety of the treatment. However, this is not the case for non fatal cardiac arrhythmias, in particular atrial fibrillation.

AF is the most common arrhythmia and affects more than 33 million people worldwide [147]. The prevalence of AF is higher in men than in women probably due to different CV risks that are gender-related [148]. In addition, polymorphic ventricular tachycardia torsades de pointe (TdP) is in some way gender-specific, being less frequent in men than in women [149]. Preclinical studies

suggested that T ablation promotes arrhythmias in aged mice by increasing late inward sodium current and prolonging repolarization in mouse ventricular myocytes [150,151]. Accordingly, androgen deprivation therapy (ADT) in patients with prostate cancer increases overall CV risk and the risk of arrhythmias, including TdP [149,152,153]. It is suggested that T shortens the action potential interval duration with a shortening of the QTc interval [151], acting through distinct signaling pathways, including inhibition of L-type calcium current, enhancing I_{Kr} and the slow component of the delayed rectifier calcium current (I_{Ks}) [154]. In an RCT three-way crossover study, transdermal T attenuates drug-induced QT lengthening in older men [155] by affecting both early and late ventricular repolarization [156].

Epidemiological studies reported conflicting results, most probably because of a U-shaped effect of androgens, as recently demonstrated [138]. In fact, analysis of 173,498 men from a UK Biobank showed that both low and high free T were associated with AF, while bradycardia was more frequent in those with low free T and the reverse was observed for ventricular arrhythmia (VA). The present meta-analysis of available epidemiological studies shows a significant association between low T, and a higher risk of AF, when an age-adjusted model is considered. The latter observation was attenuated in a fully adjusted model, where only a trend for an association between low T and AF was observed. As reported in the TRAVERSE trial [15], the present meta-analytic results show a trend towards an increased risk ($p=0.07$) of overall arrhythmia for TRT in intervention trials. However, the latter association was further blunted in studies including only hypogonadal men or excluding frail men when TRT was prescribed at dosages above the suggested recommendations. Considering that the TRAVERSE trial enrolled only frail men at high CV risk, it is conceivable that the association between TRT and arrhythmia or AF was more evident in the TRAVERSE trial than in other trials. Finally, as stated before, both non-fatal arrhythmia and AF were not trial endpoints of the TRAVERSE study but only investigator-reported adverse events [15], and hence they should be interpreted with caution. It is our expert opinion that a possible association between TRT and cardiac arrhythmias should be more deeply investigated in future

dedicated trials, with AF and other arrhythmias as end-points. Overall, the evidence here reported, although caution suggests evaluating the presence of high CV risk when prescribing TRT in hypogonadal men, as those enrolled in the TRAVERSE study, along with a baseline and follow-up ECG and/or cardiological evaluation, to screen for atrial fibrillation.

Several limitations should be recognized. Data derived from studies with non-CV primary end points should be interpreted with caution due to the lack of predefined diagnostic criteria and screening methods for incident CVD and the risk of misdiagnosis and under-diagnosis. However, our analysis shows that the final result is similar when TRAVERSE and non-TRAVERSE studies are considered. The duration of available RCTs, including the TRAVERSE study, is still limited, not allowing inferences on long-term effects. The information related to AF as derived from non-TRAVERSE studies is limited and many studies reporting data on the risk of overall arrhythmias do not clarify the specific type of event observed. In addition, the correct assessment of AF can be problematic in many studies if a baseline and post-treatment ECG was not correctly performed. Furthermore, even if an ECG was performed, it should be recognized that AF may be often intermittent and asymptomatic limiting its correct evaluation.

In conclusion, available data show that TRT is not associated with an increased risk of CV events when hypogonadism is properly diagnosed and treated. In fact, the three largest T RCTs reported to date, T Trials [13], T4DM [14], and TRAVERSE [15], showed no signal for adverse CV events, as does the present meta-analysis. The possible advantages of TRT observed in uncomplicated subjects could be blunted when TRT is used in patients at high CV risk. Considering that the majority of subjects involved in the present meta-analyzed trials are classified as having *functional* HG, it is difficult to extend this CV safety signal to other forms of hypogonadism, including the genetic forms. For instance, in the most common genetic condition, Klinefelter's Syndrome, TRT was not able to restore the levels of control subjects, altered body composition and glycol-metabolic parameters, as recently reported in a meta-analysis of observational studies [157].

Age-dependent decline of T observed in aging men (the so-called *functional* hypogonadism) is the result of a combination of genetic background, accumulation of morbidities, and wrong lifestyle behaviors [10,158,159]. Hence, correcting unsafe lifestyle behavior is mandatory in these hypogonadal subjects. Other studies are advisable to better clarify whether or not early identification of hypogonadism and an early TRT approach can reduce long-term CV risk.

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Source	#	MH-OR	LL	UL	p
AMI					
Overall	18	0,76	0,40	1,44	0,40
Traverse	1	1,10	0,78	1,56	0,94
Acute coronary syndrome					
Overall	22	0,70	0,43	1,16	0,17
Traverse	1	1,17	0,96	1,44	0,13
Stroke					
Overall	13	1,00	0,68	1,46	1,00
Traverse	19	1,01	0,74	1,37	0,94
Heart failure					
Overall	8	0,75	0,30	1,86	0,53
Traverse	1	1,10	0,75	1,62	0,62
CV mortality					
Overall	17	1,11	0,53	2,35	0,78
Traverse	1	0,84	0,63	1,12	0,24
MACE					
Overall	35	0,89	0,63	1,28	0,54
Traverse	1	1,05	0,90	1,23	0,52

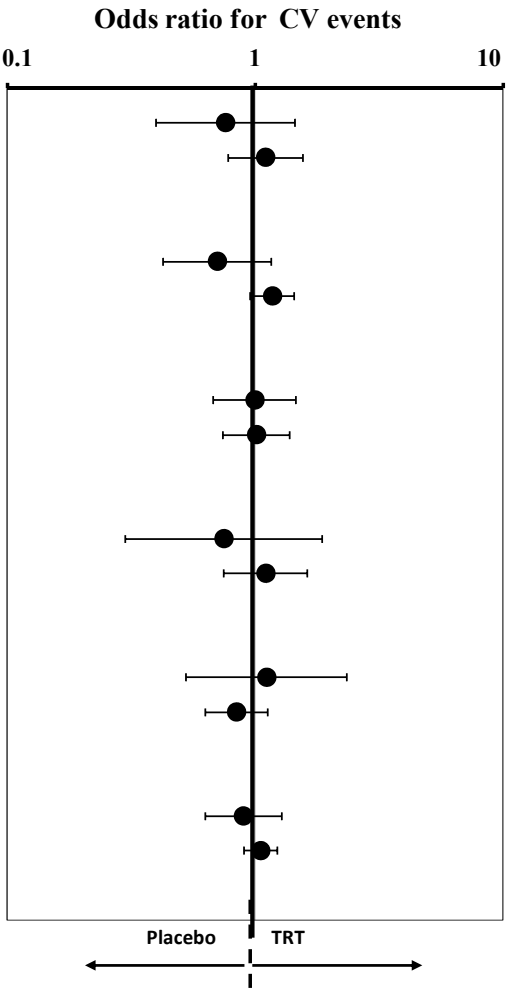

Figure 1. MH-Odds ratio Odds ratio for acute myocardial infarction (AMI), acute coronary syndrome, stroke, heart failure, cardiovascular (CV) mortality, and overall major adverse CV events (MACE) in subjects treated with testosterone (TRT) or placebo.

Source	#	MH-OR	LL	UL	p
Arrhythmias					
Overall	15	1,75	0,96	3,16	0,07
Traverse	1	1,57	1,19	2,07	0,00
Arrhythmias					
Overall (total T < 12 nmol/L)	8	1,73	0,78	3,80	0,17
Overall (frail men excluded)	13	1,61	0,84	3,08	0,15
Atrial Fibrillation					
Overall	5	1,76	0,61	5,06	0,30
Traverse	1	1,46	1,06	2,02	0,02
Atrial Fibrillation					
Overall (total T < 12 nmol/L)	3	4,10	0,66	25,37	0,13
Overall (frail men excluded)	4	1,44	0,46	4,46	0,53

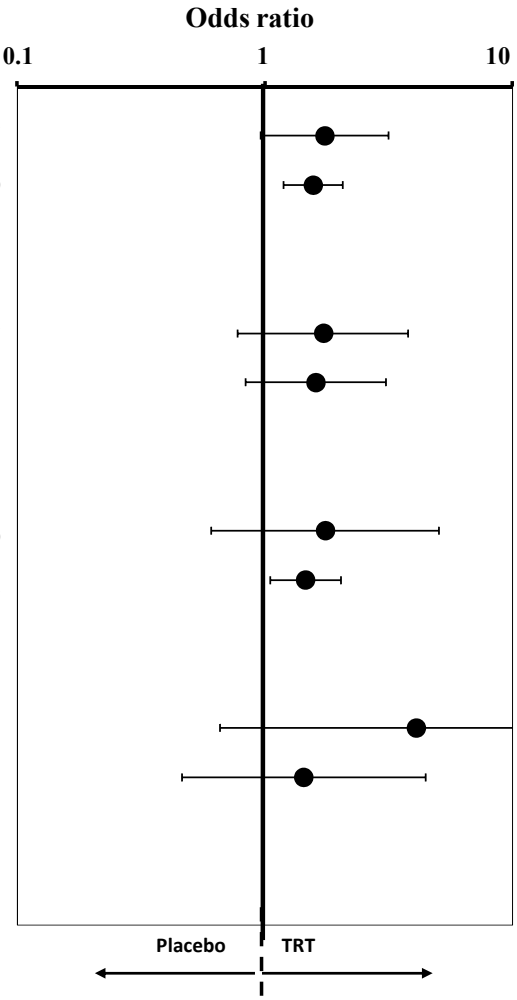

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Figure 2. Mantel-Haenszel odds ratio (MH-OR: Odds ratio) ratio for incident overall arrhythmias or atrial fibrillation in subjects treated with testosterone (TRT) or placebo. LL: Lower limit; UL: Upper limit.

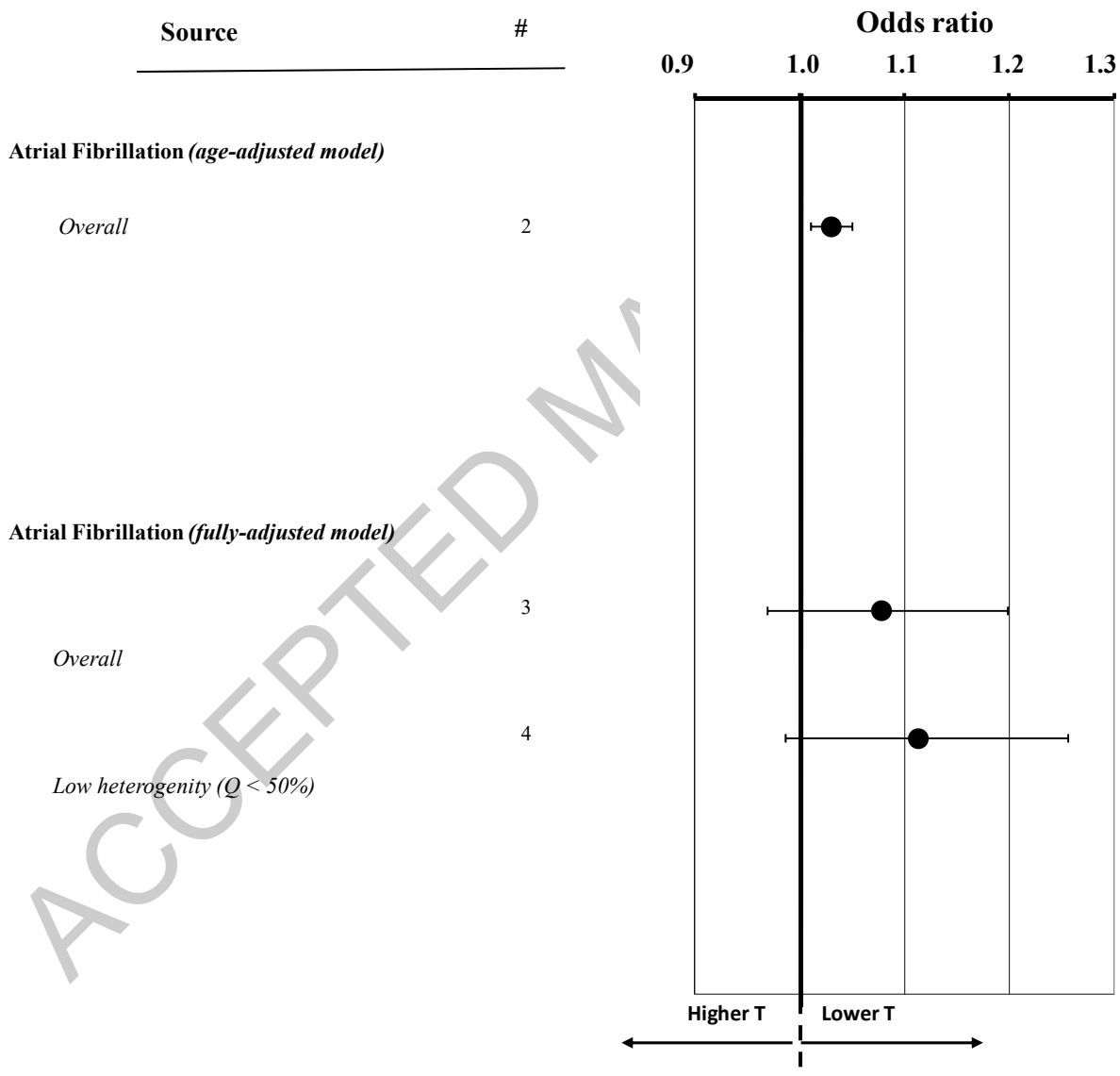

Figure 3. Mantel-Haenszel odds ratio (MH-OR: Odds ratio) ratio for incident atrial fibrillation according to testosterone circulating level as derived from population-based studies. LL: Lower limit; UL: Upper limit.

y (ref.)	# patients (T/placebo)	Trial duration (weeks)	Age (years)	Comorbidities	Baseline TT (nMl)	BMI (kg/m^2)	T levels	Dose
o, 1986 (32)	134/87	112	53,0	Alcoholic cirrhosis *	-	24.6	Mixed	micronized T 6
	8/10	26	-	Institutionalized men*	-	29.6	TT < 12 nM	TE 150 mg/70 k
	20/18	8	38,9	Elderly men	-	-	Eugonadal	TU 120 m
	17/18	39	60,8	Rheumatoid arthritis	15,9	-	Mixed	TE 250 mg
	17/18	13	-	HIV	-	-	Mixed	TC 200 mg/2
	17/15	26	66,4	Elderly men	9,1	28.3	TT< 12 nM	TC 200 mg/2
	20/21	12	-	HIV	8,0	23.5	TT< 12 nM	T patch 5 m
9)	26/25	26	42,0	HIV	10,7	21.5	Mixed	TE 300 mg/3
	7/7	12	66,7	Elderly men	11,5	27.4	Mixed	TE 200 mg/2
	11/10	12	27,0	Healthy men in training	-	26.1	Eugonadal	TE 3,5 mg/kg
	54/54	156	73,1	Elderly men	12,6	27.1	Mixed	T patch 6 m
	17/14	16	41,3	HIV	6,7	24.7	TT< 12 nM	TE 100 mg
	25/25	12	62,0	Chronicstable angina	12,9	28.1	Mixed	T patch 5 m
5)	27/27	12	41,9	HIV	22,8	-	Eugonadal	TE 200 mg
	39/35	6	39,0	HIV	13,2		Mixed	TC 200 mg/2
	21/17	26	70,0	Elderly men	13,8	26.1	Eugonadal	TE 100 mg/2
	16/19	26	40,9	Cytotoxic chemotherapy*	13,3	26.9	Mixed	T patch 2.5-5
	13/17	6	51,4	Major depressivedisorder	9,1	-	TT< 12 nM	TE 200 mg
	6/6	12	53,1	LOH	8,7	28.6	TT< 12 nM	TG 125 m
	12/13	4	70,4	Elderly men	12,8	-	Mixed	TE 600 mg
2)	21/17	26	70,0	Elderly men	14,0	26.9	Mixed	TE 100 mg/2
	7/5	26	-	Elderly men	11,3	-	Mixed	TE 100 mg
	17/17	4	67,0	Elderly men	9,6	28.0	Mixed	Combination of T phenylpropi isocaproat T decano 500-250-250 mg
	33/6	12	-	Elderly men	8,9	-	Mixed	TU 160 m
	12/10	8	49,2	Refractory depression	9,6	30.9	TT< 12 nM	TG 100 m
	307/99	12	56,8	Sexual dysfunction	8,0	30.1	TT< 12 nM	TG 100 m
	5/5	52	70,7	Alzheimer's disease	-	-	TT< 8 nM	TE 200 mg/2
	24/24	156	71,0	Elderly men	10,2	28.3	TT< 12 nM	TE 200 mg/2
	40/45	52	66,0	Elderly men	10,2	-	TT< 12 nM	TU 160 m
	6/5	12	79,6	Mild cognitive loss	13,8	-	Mixed	TE 200 mg/3
	5/5	4	60,8	Ischemic heart disease	4,2	30.9	Eugonadal	Combination of T phenylprop T isocaproa T decano 100 mg/2
	29/29	4	61,6	Elderly men	4,4	31.3	TT< 8 nM	Combination of T phenylprop T isocaproa T decano 100 mg/2
	38/39	8	41,0	HIV	20,4	-	Mixed	TC 400 mg/2
)	15/14	26	66,5	COPD	21,1	24.5	Eugonadal	TE 250 mg/
	13/13	6	46,4	Refractory depression	14,7	-	TT< 12 nM	TE 200 mg
	37/34	12	78,2	Elderlyfrailmen*	10,7	26.2	Mixed	TE 100 mg
06 (68)	19/21	26	55,8	Dialysis subjects*	7,2	-	TT< 12 nM	TG 100 m
9)	23/20	26	69,9	Elderly men	16	26.8	Mixed	T patch 5 m
	66/80	12	39,9	HIV	25,7	20.5	Eugonadal	Combination of T phenylprop T isocaproa T decano

								250 mg/2 v
	27/27	26	63,2	T2DM	8,6	33.3	TT< 8 nM	Combination of T T phenylprop T isocaproa T decano 200 mg/2 v
2)	17/17	12	72,0	Elderly men	14,1	27.6	TT< 12 nM	T patch 5 m
	9/9	24	69,8	Mild Alzheimer disease	12,2	-	Mixed	TG 75 mg
	14/15	24	62,3	Elderly men	13,0	-	Mixed	TG 75 mg
	37/39	52	64,0	Heart failure	13,0	27.8	Mixed	T patch 5 m
	22/22	26	69,0	Elderly men	8,1	28.9	TT< 12 nM	TE 150 mg/2
	20/19	26	61,4	Elderly men	8,0	-	TT< 12 nM	T patch 5 m
	30/32	104	66,7	Elderly men	13	27.9	Mixed	T patch 5 m
	15/15	26	68,3	Parkinson disease	11,1	-	Mixed	TE 200 mg
	44/44	24	45,5	HIV	13,9	26.6	Mixed	TG 100 mg
	20/18	12	-	Elderly men	8,1	-	TT< 12 nM	TG 50 mg
	13/13	52	69,1	Elderly men	8,4	25.8	TT< 12 nM	TU 1000 mg/1
	31/31	52	63,3	Elderly men	14,1	30.0	Mixed	T patch 5 m
	25/23	52	63,2	Elderly men	10,5	27.3	TT< 12 nM	TE250 mg/3
08 (84)	120/117	26	67,3	Elderly men	10,7	27.4	Mixed	TU160 mg
	30/31	17	43,2	HIV	14,6	24.9	Mixed	TE3 00 mg
)	19/19	52	69,0	Elderly men	8,3	29.6	TT< 12 nM	TU 1000 mg/1
	35/35	12	70,0	Heart failure	7,5	26.3	Mixed	TU 1000 mg/1
)	6/6	52	76,0	Elderly frail men*	20,3	19.8	Mixed	TU160 mg
	20/20	13	-	Erectile dysfunction	6,2	-	Mixed	TG 50 mg
	237/79	52	58,7	Elderly men	12,8	27.3	Mixed	TU 80-240m
	7/6	52	64,8	Stable chronic angina	9,9	-	TT< 12 nM	TU 1000 mg/1
	13/10	6	50,6	Dysthymia	11,6	32.0	Mixed	TC200 mg/1
	17/16	12	59,3	Dysthymia	10,1	33.1	Mixed	TG 75 mg
	40/10	104	57,8	MetS and/or T2DM	8,5	30.4	TT< 12 nM	TU 1000mg/1
	42/10	52	57,2	MetS and/or T2DM	7,4	30.4	TT< 12 nM	TU 160 mg/day/ T weeks
	106/103	26	74	Elderly frail men*	8,3	29.9	TT< 12 nM	TG 100 mg
	43/44	12	68,7	Chronic stable angina	-	27.3	Mixed	TU 120 mg
	11/11	26	44,2	T2DM	10,1	23.9	TT< 12 nM	TC 200 mg/2
98)	113/71	30	52,1	MetS	7	34.9	TT< 12 nM	TU 1000mg/1
010 (99)	136/138	26	73,8	Elderly frail men*	11	27.8	Mixed	TG 50 mg
	50/50	6	51.1	Major depressive disorder	11.8	-	Mixed	TG 50-100 m
	60/60	48	53,2	Elderly men	9	29.3	Mixed	TU 1000mg/1
	108/112	52	59,9	MetS and/or T2DM	9,5	32.1	Mixed	TG 60 mg
3)	234/40	26	53,9	Elderly men	9,7	23.5	TT< 12 nM	TG 12.5-50m
	183/179	48	62,0	Elderly men	10,5	28.6	Mixed	TG 50-75 m
105)	20/18	24	67.5	Elderly men	12.6	30.2	Mixed	TG 50 mg
	33/34	18	48,5	Obese with OSA	13,3	35.8	Mixed	TU 1000mg/1
	92/98	30	61,5	T2DM	9,0	32.7	TT< 12 nM	TU 1000mg/1
)	96/47	52	66,5	Elderly men	10,2	-	TT< 12 nM	TG 100mg
	43/24	156	71,8	Elderly men	13,4	25.4	Mixed	T patch 6 m
	9/8	20	69,6	Elderly men	-	-	Mixed	TE 100 mg/
	14/16	52	70,5	Elderly men	8,8	29.9	TT< 10,4 nM	TE 125 mg
)	45/43	40	62,0	T2DM	8,6	32.4	< 12 nM	TU 1000 mg/1
)	43/42	24	49,7	T2DM	-	35.8	Mixed	TE 250/12
	25/25	24	-	Elderly men	17,2	-	Mixed	T cream 5% 5
	155/151	156	67,6	Elderly men	10,5	32.6	Mixed	TG 75 mg
	36/29	14	48,9	Treated with opioids	8,5	28.1	TT< 12 nM	TG 50 mg
)	10/12	24	70,5	Elderly men	10,6	-	TT< 10,4 nM	TG 50-100 m
7)	23/23	24	67.5	Elderly men	-	-	BT < 7.3 nM	TG 50-100 m
	40/36	16	50,7	Ejaculatory dysfunction	7,5	30.7	TT< 10,4 nM	T solution 60
	358/357	12	55,3	Elderly men	6,9	30.6	TT< 10,4 nM	T solution 60-1
9)	22/22	23	54,7	T2DM	8,6	39.9	FT <225 pM	TC 200 mg/2
21)	20/19	24	60.0	T2DM	8.2	30.7	BT < 7.3 nM	TG 50 mg
)	395/395	52	72,2	Elderly men	8,0	31.0	TT< 9.5 nM	TG 50-100 m
	88/82	52	71,2	Elderly men	8,2	30.5	TT< 9.5 nM	TG 50 mg
4)	6/7	22	46,3	T1DM	10,9	32.7	TT< 10 nM	TU 1000 mg/1
	28/27	52	60.2	T2DM	7.6	33.3	TT< 11 nM	TU 1000 mg/1

	13/11	24	64.9	End stage renal disease	15.2	22.1	Mixed	TE 250/2 v
2018 (127)	14/12	52	65.5	Heart failure	8.2	30.0	TT< 11 nM	TU 1000 mg/1
	68/68	26	-	Cancer survivors	-	27.9	TT < 12 nM	TG 60 mg
	42/42	6	60.0	Erectile dysfunction	10.8	31.7	Mixed	TG 75 mg
0)	42/41	24	72.9	Elderly men	7.5	37.0	TT < 10.4 nM	TG 40.5 m
	18/20	24	54.5	Treated with opioids	7.1	30.9	TT < 12 nM	TU 1000 mg/1
	504/503	108	59.7	T2DM or obesity	13.7	34.7	Mixed	TU 1000 mg/1
2)	35/34	52	43.0	Cancer survivors	15.1	25.9	Eugonadal	TG 10mg
	7/7	12	68.0	Elderly men	11.3	26.5	Mixed	T cream 5% 10
	2601/2603	86.8	63.3	High CV risk	7.8	34.9	TT < 10.4 nM	TG 40.5mg

Table 1. Characteristics of the randomized, placebo controlled clinical studies included in the meta-analysis. § Subjects with Alzheimer disease. TT=total testosterone, FT= free testosterone; BT= bioavailable testosterone TE= testosterone enanthate, TU= testosterone undecanoate; TC=testosterone cypionate. TG= testosterone gel; NR= not reported; HIV=human immunodeficiency virus; LOH= late onset hypogonadism; COPD=chronic obstructive pulmonary diseases; T2DM= type 2 diabetes mellitus; MetS=metabolic syndrome; BPH=benign prostatic hyperplasia; OSA=obstructive sleep apnea; *considered as frail men;

Study	**#**	**Population**	**Follow-up (years)**	**Testosterone cut-off**	**Age (years)**	**BMI (kg/m^2)**	**Active smokers (%)**	**DM (%)**	**TT (nmol/L)**
Magnani et al., 2014 (134)	1251	Framingham Heart Study (US)	10	Each standard deviation decrease	68.0	26.7	21	11	15.43
O'Neal et al., 2017 (135)	3003	The Multi-Ethnic Study of Atherosclerosis (US)	10.9	173498	61.0	28.0	14.0	15.0	14.9
Zeller et al., 2018 (136)	3876	FINRISK97 (Finland)	13.8	Each T nanomolar decerase	49.2	26.6	26.3	5.7	17.1
Berger et al ., 2019 (137)	4224	Atherosclerosis Risk in Communities (ARIC) study (US)	13.7	Highest vs lowest quartile	63.0	28.0	14.0	17.0	-
Xu et al., 2023 (138)	173498	UK biobank	11.8	IV vs III quartile	56.0	27.2	12.6	-	11.67

Table 2. Descriptive characteristic of the available population-based studies evaluating the relationship between baseline T levels and incident atrial fibrillation. TT= total testosterone; - = not reported; BMI= body mass index; DM= diabetes mellitus; US=United States of America; UK=United Kingdom.