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REVIEW ARTICLE

Effectiveness of testosterone therapy in hypogonadal patients and its controversial adverse impact on the cardiovascular system

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ABSTRACT

Testosterone is the major male hormone produced by testicles which are directly associated with man's appearance and secondary sexual developments. Androgen deficiency starts when the male hormonal level falls from its normal range though, in youngsters, the deficiency occurs due to disruption of the normal functioning of pituitary, hypothalamus glands, and testes. Thus, testosterone replacement therapy was already known for the treatment of androgen deficiency with lesser risks of producing cardiovascular problems. Since from previous years, the treatment threshold in the form of testosterone replacement therapy has effectively increased to that extent that it was prescribed for those conditions which it was considered as inappropriate. However, there are some research studies and clinical trials available that proposed the higher risk of inducing cardiovascular disease with the use of testosterone replacement therapy. Thus under the light of these results, the FDA has published the report of the increased risk of cardiovascular disease with the increased use of testosterone replacement therapy. Nevertheless, there is not a single trial available or designed that could evaluate the risk of cardiovascular events with the use of testosterone replacement therapy. As a result, the use of testosterone still questioned the cardiovascular safety of this replacement therapy. Thus, this literature outlines the distribution pattern of disease by investigating the data and link between serum testosterone level and the cardiovascular disease, also the prescription data of testosterone replacement therapy patients and their tendency of inducing cardiovascular disease, meta-analysis and the trials regarding testosterone replacement therapy and its connection with the risks of causing cardiovascular disease and lastly, the possible effects of testosterone replacement therapy on the cardiovascular system. This study aims to evaluate the available evidence regarding the use of testosterone replacement therapy when choosing it as a treatment plan for their patients.

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Introduction

T is the major male hormone produced by testicles which are directly associated with man's appearance and secondary sexual developments. It also regulates fertility in males. Hypogonadism in males is a condition when sex hormones are not produced by sex glands. Thus, hypogonadism can be categorized into two types. Primary hypogonadism in which testes does not produce enough sex hormones while secondary hypogonadism is the condition in which hypothalamus and pituitary glands are not working correctly (Snyder and Lawrence 1980). Testosterone replacement therapy is considered harmless in youngsters with hypogonadism in which serum testosterone level is deficient (Seftel 2019). However, ageing is also a contributing factor of decreasing testosterone level in males (Wu et al. 2008; Bhasin et al. 2011) even in the absence of pituitary or testicular disease (Harman et al. 2001; Shores et al. 2006). From studies, it was evident that prevalence of lower level of testosterone was seen more deliberately in the ages between 50 and 80 years come up with different percentages of 12–49%, respectively (Harman et al. 2001).

In many cases, the testosterone level is low that it seems to touch the lower range. Thus, these cases did not show the significant features of hypogonadism, and its symptomatic prevalence is about ∼2% (Liu et al. 2016), therefore as a result the outcomes and the risks related to testosterone replacement therapy is still obscure in those men in which the age-related testosterone deficiency is seen. When a clinical trial was conducted in aged persons in which testosterone replacement therapy was done, it could be observed that the mood and sexual activity of these individuals improved. In contrast, it did not show any improvement in body strength to perform necessary activities (Synder et al. 2016). Besides with a limited number of outcomes, the unapproved use of testosterone therapy has increased all over the world between middle to an aged group of individuals (Handelsman 2004; Baillargeon et al. 2013; Layton et al. 2014; Nguyen et al. 2015). After that, the mass media has started a campaign (Handelsman 2017), which resulted in the declaration of safety warning by FDA in 2014 to cut down the prescription rate of testosterone therapy by making people aware about the cardiovascular risks associated with testosterone replacement therapy (Baillargeon et al. 2018).

There are several articles and studies available that predict the connection between decreased testosterone and increased cardiovascular risk (Carrero et al. 2008; Laughlin et al. 2008; Yeap et al. 2009, 2014; Haring et al. 2010; Ohlsson et al. 2011; Soisson et al. 2013). It was predicted that any kind of mild or severe disease could result in a low level of testosterone; hence it could say that the individuals with cardiovascular disease had low testosterone level due to its illness yet it could not be the cause of illness. This decreased testosterone level is the natural response of the body to behave as a biomarker of disease in the body. On the other hand, in contradict to the cohort studies, the data collected from prescription showed higher cardiovascular risks with the use of testosterone replacement therapy (Page 2014; Etminan et al. 2015; Tan et al. 2015; Martinez et al. 2016). In contrast, the same study conducted by different research group reported testosterone replacement therapy as beneficial or no risk factor (Shores et al. 2012; Muraleedharan et al. 2013; Baillargeon et al. 2014, 2015; Li et al. 2015; Sharma et al. 2015, 2016, 2017; Tan et al. 2015; Anderson et al. 2016; Wallis et al. 2016; Cheetham et al. 2017; Oni et al. 2017). The results achieved from various research studies indicated the higher risks of cardiovascular events with the use of testosterone replacement therapy (Hall 2010; Xu et al. 2013). However, this increased rate of cardiovascular problems has started the discussion of cardiovascular safety from testosterone replacement.

There are no data available or trials conducted to evaluate the risk of inducing cardiovascular events due to testosterone replacement therapy. Still, the associated risks of cardiovascular events with testosterone replacement therapy are obscure. When a TRAVERSE trial (US National Library of Medicine 2019) is conducted with testosterone gel applied transversally in comparison with placebo gel to evaluate the cardiovascular risks in 2018 and mapped five years' plan.

However, the conclusions achieved from data will take a decade before its publication. In this context, the review will help clinicians to inform about the said risks associated with testosterone replacement therapy. This literature demonstrated the connection between testosterone level in serum and the cardiovascular risks associated with it. It also discusses the epidemiological progression of cardiovascular mortality and other safety issues produced due to testosterone replacement therapy in men. Several different trials, such as randomized or controlled trials are conducted to evaluate the cardiovascular risks due to testosterone therapy. Similarly, preclinical trials included meta-analyses in randomized trials, and the mechanistic studies are also carried out to predict the effect of testosterone in the induction of cardiovascular problems.

Epidemiological assessment of the influence of endogenously produced testosterone level

On studying the epidemiological behavior, it was evident that lower the level of endogenous testosterone in the body, higher the risks of cardiovascular events associated with that. These events include cardiovascular mortality and all-cause death in aged men. This research reviewed the epidemiological distribution of benefits linked with cardiovascular events and also the authentic evaluation patterns available to measure the endogenous testosterone level.

Association between endogenous testosterone level and cardiovascular risks

Tables 1 and 2 explain the series of cohort studies conducted to find out the link between endogenous testosterone level in men and the cardiovascular events associated with that. The detailed study of this research is reviewed after applying some limitation. The first limitation is the use of immunoassay for measurement of testosterone level, which has lesser accuracy specifically at lower levels of testosterone (Wang et al. 2004; Sikaris et al. 2005) as compared to the other method of testosterone measurement such as gold-standard methods (Handelsman and Wartofsky 2013). The other limitation is measuring only a single T. However, its level shows a diurnal variation in serum. The last limitation is the consideration of only testosterone levels in serum without considering the androgen and its deficiency.

When the study was conducted on Australian men in the age group of greater than 70 years and persuade for about 3.5 years for the reason of low testosterone level viz. <337 ng/dl when calculating based on immunoassay have higher chances of inducing cardiovascular events such as ischemic attack or stroke as compared with the normal levels of testosterone in the serum (Ohlsson et al. 2011). However, the above findings were further established with the help of mass spectrometry (Yeap et al. 2014). On the contrary, 1032 aged individuals did not experience any cardiovascular event due to low testosterone level as confirmed by the Cardiovascular Health Study (Shores et al. 2014). Still, the reason behind this contradiction is undecided. It can be said the variation in epidemiological behavior and different life events may cause a change in a statement. When discussing the ARIC study, it was found that 1558 aged men presented with ischemic stroke were not linked with testosterone replacement therapy in 14.1 years. Another captivating thing about testosterone replacement therapy is found when studied in Swedish men with an age group of 69–81 years that it behaved as a protective effect against different cardiovascular events such as angina, cardiovascular revascularization, or death. It was also found that an increased level of testosterone viz. $\geq$550 ng/dl reduced its rate of cardiovascular risk, up to 30% (Yeap et al. 2009). A French Three-City prospective cohort study (Soisson et al. 2013) conducted on 495 men in age group more significant than 65 years reported a j shaped link among arterial disease and testosterone level in which 34 patients were experiencing a stroke, and 112 patients experience coronary heart disease in the 4-year duration of persuasion. However, these risk factors of cardiovascular events were regulated for the safety purpose. It is also found that testosterone level at its highest viz. HR 3.61, 95% CI 1.55–8.45 and lowest viz. HR 2.23, 95% CI 1.02–4.88 have higher chances of inducing ischemic arterial disease as compared to those men whose testosterone level range was found in quintile (Soisson et al. 2013). These results concluded the fact that testosterone levels in a particular range can provide a cardioprotective effect as compared to its extreme values. It was found that lower range of testosterone results in atrial fibrillation in middle and aged men. When a cohort study was conducted under Framingham Heart Study (Magnani et al. 2014) on 1251 men in the age range of 68 years, it was found on persuasion that 275 men developed atrial fibrillation.

The connection between testosterone levels and age was reported in adjusted hazard models. According to this model in men aged between 55 and 69 years, showed a decrease in standard deviation in serum testosterone level, which is associated with hazard ratio viz. 1.30 (95% CI 1.07–1.59). While, on the other hand, if the age limit reached up to 80 years, the hazard ratio also increases viz. 3.53 (95% CI 1.96–6.37) (Magnani et al. 2014). According to the Cardiovascular Health Study (Rosenberg et al. 2018) and FINRISK97 (Zeller et al. 2018), decreased level of androgen is directly connected with events of atrial fibrillation in men.

A population-based cohort study conducted in 2011 analyzed 19 prospective benefits as well as a nested case-control study assesses the link between endogenous testosterone level and cardiovascular actions such as IHD, MI, and stroke. Still, it was also found that the connection between endogenous testosterone and CVD is weak if the testosterone level is high in serum thus the relative risk (RR) was 0.89, 95% CI 0.83–0.96 (Ruige et al. 2011). This analysis also documented that if the age of men is less than 70 years, then there was not any communication between endogenous testosterone and cardiovascular (Ruige et al. 2011). Mendelian randomization of cohort studies conducted on 1882 men (range $20-79$ years) who took part in Study of Health in Pomerania reported that there is not any fundamental relationship among testosterone and cardiovascular actions and mortality (Haring et al. 2013a, 2013b). It might result from

Table 1. Studies to claim a direct correlation between endogenous androgen levels and cardiovascular risk.

Reference	Study subjects	Conclusion
Magnani et al. (2014)	1251 subjects (age: ≥55) were studied for 10 years ending in 2014.	The lower levels of testosterone and estradiol were found associated with the incidence of atrial fibrillation (HR = 3.53 (CI_{95} 1.96–6.37)).
Yeap et al. (2014)	3690 subjects (age: 70–89) were studied for 9 years ending in 2014.	Lower testosterone and dihydrotestosterone levels were found associated with all-cause deaths (HR = 0.82, $p = 0.033$). Higher dihydrotestosterone levels were found associated with lower mortality risk due to ischemic heart diseases.
Yeap et al. (2014)	3690 subjects (age: 70–89) were studied for 6.6 years ending in 2014.	Higher levels of testosterone or dihydrotestosterone were associated with a lower risk of stroke. The hazard ratio for testosterone was found to be 0.56 (CI_{95} 0.39–0.81). Besides, the study claimed no association between myocardial infarction and the higher levels of testosterone.
Soisson et al. (2013)	491 subjects (age: ≤65) were studied for 4 years ending in 2013.	Plasma testosterone levels in their optimal physiological range are found to be cardioprotective since a deviation from this physiological range in both directions has resulted in a higher incidence of ischemic arterial disease. HR values for the testosterone levels above and below the optimal range were 3.61 (CI_{95} 1.55–8.45) and 2.23 (CI_{95} 1.02–4.88), respectively.
Tan et al. (2015)	581 subjects (age: 31–88) were studied for 5.8 years ending in 2013.	The all-cause mortality rate was higher in men with low testosterone levels HR = 2.02 (CI_{95} 1.2–3.4)
Romanò (2001)	3637 subjects (age: 70–88) were studied for 5.1 years ending in 2001.	Lower free testosterone levels were associated with cardiovascular related mortalities (HR = 1.71 (CI_{95} 1.12–2.62)) and all-cause mortality (HR = 1.62 (CI_{95} 1.20–2.19)).
Ohlsson et al. (2011)	2416 subjects (age: 69–81) were studied for 5 years ending in 2011.	Higher plasma testosterone levels (≥550 ng/dl) were found inversely associated with the incidence of cardiovascular disease (HR = 0.70 (CI_{95} 0.56–0.88)).
Mathur et al. (2009)	930 subjects (age: ≤60) were studied ending in 2008.	High all-cause and vascular mortalities were recorded in patients with coronary artery disease having low levels of bioavailable testosterone (HR = 2.27 (CI_{95} 1.45–3.6)).
Webb et al. (2008)	1114 subjects (age: ≥20) were studied for 18 years ending in 2007.	Low levels of free testosterone (HR = 1.43 (CI_{95} 1.09–1.87)) and bioavailable testosterone (HR = 1.52(CI_{95} 1.15–2.02)) were found associated with a higher incidence of all-cause mortalities.
Tan et al. (2015)	1954 subjects (age: 20–79) were studied for 7.2 years ending in 2014.	Cardiovascular and the all-cause mortality rates were found associated with lower total testosterone levels. The HR for cardiovascular and all-cause mortalities was 2.56 (CI_{95} 1.15–6.52) and 2.32 (CI_{95} 1.38–3.89), respectively.
Yeap et al. (2009)	3443 subjects (age: ≥70) were studied for 3.5 years ending in 2009.	Lower testosterone levels were found associated with the transient ischemic attack and cerebral stroke.
Smith et al. (2001)	1568 subjects (age: average 59.6) were studied for 11.2 years ending in 2000.	The high all-cause mortality rate was associated with lower levels of free testosterone levels (HR = 1.24 (CI_{95} 1.01–1.53)).
Dockery et al. (2009)	3014 subjects (age: 69–80) were studies for 4.5 years ending in 2009.	Higher all-cause mortalities were recorded in patients with lower total testosterone levels (HR = 1.65 (CI_{95} 1.29–2.12)).
Haring et al. (2010)	794 subjects (age: 50–91) were studied for 11.8 years ending in 2008.	Cardiovascular and the all-cause mortality rates were found higher in patients with lower total testosterone levels. The HR values for cardiovascular and all-cause mortalities were 1.38 (CI_{95} 1.02–1.85) 1.40 (CI_{95} 1.14–1.71), respectively.
Laughlin et al. (2008)	11 606 subjects (age: 40–79) were studied for 7 years ending in 2007.	Higher cardiovascular and all-cause mortalities were found associated with lower levels of total testosterone levels. In an order of increasing testosterone levels, the odds ratios were 0.75 (CI_{95} 0.55–1.00), 0.62 (CI_{95} 0.45–0.84), and 0.59 (CI_{95} 0.42–0.85) compared with the lowest quartile.
Johannsson et al. (2005)	858 subjects (age: ≤40) were studied for 4.3 years ending in 2004.	The higher mortality rate was associated with lower total testosterone levels (HR 1.88 (CI_{95} 1.34–2.63)).
Stramba-badiale et al. (1995)	182 747 subjects (age: ≥66) were studied for 5.1 years ending in 1995.	Androgen deprivation therapy was found associated with increased risk of venous thromboembolism (HR = 1.10 (CI_{95} 1.04–1.15)) and peripheral artery disease (HR = 1.16 (CI_{95} 1.12–1.21)).

Table 2. Investigations claiming an indirect (androgen deprivation therapy in prostate cancer patients) correlation between endogenous androgen levels and cardiovascular risk.

Reference	Study subjects	Conclusion
Alimurung et al. (1950)	5103 subjects (age: 50–84) were studies for 6 years.	Combination therapy of luteinizing hormone-releasing hormone receptor antagonists and bicalutamide (anti-androgen) was associated with increased risk of coronary heart disease (HR = 4.35 (CI_{95} 1.94–9.75)), acute myocardial infarction (HR = 3.57 (CI_{95} 1.44–8.86)), and heart failure (OR = 3.19 (CI_{95} 1.10–9.27)).
Rautaharju et al. (1992)	22 310 subjects (age: ≥40) were studied for 3.9 years.	Androgen deprivation therapy was associated with the risk of transient ischemic attack (RR = 1.18 (CI_{95} 1.00–1.39)).
Tep-Areenan et al. (2002)	37 443 subjects (age: average 66.9) were studied for 5 years. Androgen	Deprivation therapy was associated with higher risk of diabetes (aHR = 1.28 (CI_{95} 1.19–1.38)) incident coronary heart diseases (aHR = 1.19 (CI_{95} 1.10–1.28)), myocardial infarction (aHR = 1.28 (CI_{95} 1.08–1.52)), sudden cardiac death (aHR = 1.35 (CI_{95} 1.18–1.54)), and stroke (aHR = 1.22 (CI_{95} 1.10–1.36)).
Perusquía et al. (2015)	73 196 subjects (age: ≥66) were studied for 9 years. Androgen	Deprivation therapy was associated with increased risk of incident diabetes (aHR = 1.44; $p < 0.001$), coronary heart disease (aHR = 1.16; $p < 0.001$), myocardial infarction (aHR = 1.11; $p = 0.03$) and sudden cardiac death (aHR = 1.16; $p = 0.004$).

Table 3. Studies to claim no association between cardiovascular diseases and endogenous testosterone levels in elderly men.

Reference	Study subjects	Conclusion
Buonanno et al. (1982)	A study covering 1558 men from 51 to 76 years of age.	No association was found between the incidence of atherosclerosis-related brain stroke and the endogenous testosterone levels.
Gagliano-Jucá et al. (2018a, 2018b)	A study in mean conducted in 2016.	The cardiovascular or all-cause mortality risk was not associated with total testosterone levels.
Hanley et al. (1989)	A study covering 1032 men of 66 years of age and above.	A nonlinear association between the levels of dihydrotestosterone and stroke risk was found. However, total and free testosterone levels were not associated with stroke incidence.
Zhang et al. (2011)	A study in mean conducted in 2010.	The cardiovascular or all-cause mortality risk was not associated with total testosterone levels.
Noseworthy et al. (2012)	A study on 254 men with an average age of 75.5 years in 2010	The cardiovascular morbidities, including infarction, coronary insufficiencies, and congestive cardiac failure, were not found associated with total testosterone levels.
Noseworthy et al. (2012)	A study in mean conducted in 2011	All-cause mortality risk was not found associated with higher baseline total testosterone levels at 10 years follow up period.
Smith et al. (2001)	A study in 2009 on 1318 men with an average age of 59.6 years.	Non-significant relation between the incidence of myocardial infarction and the total and free testosterone levels.
Nielsen et al. (2014)	A study in mean conducted in 2012.	The all-cause mortality risk was not associated with the total testosterone levels.
Merz et al. (1996)	A study covering 2197 men with age ranging from 71 to 94 years.	No association between the incidence of stroke and total testosterone levels was reported.
Salem et al. (2018)	A study in mean conducted in 2007.	The all-cause mortality risk was not associated with the total testosterone levels.
Hayes et al. (2013)	A study covering 2084 men with ages between 30 and 60 years.	The incidence of cardiovascular problems including angina, coronary abnormalities, stroke was not found associated in 2006 with the total testosterone levels.

residual confounding or even reverse causation. The results achieved from this review are following European Male Ageing Study (Wu et al. 2008), according to that there is not any primary connection between endogenous testosterone and age of men even after amending the confusing factors involved in it, these include use of alcohol, smoking status, and BMI of men.

Association between endogenous testosterone level and mortality

The given cohort study describes the mortality due to cardiovascular events or due to other causes and their link with testosterone level in the serum as in Table 3. Majority of the studies explained the preventative action of testosterone (Shores et al. 2006; Carrero et al. 2008; Laughlin et al. 2008; Tivesten et al. 2009; Vikan et al. 2009; Haring et al. 2010; Malkin et al. 2010; Muraleedharan et al. 2013) while some of the reviews did not find this kind of action (Araujo et al. 2007; Szulc et al. 2009; Haring et al. 2013a, 2013b; Shores et al. 2014; Chan et al. 2016). To explain this, a nested case-control study conducted on 2314 men (40–79 years) in EPIC-Norfolk study documented that the individuals without any specific disease such as cardiovascular disease or cancer it came up with high endogenous testosterone level had lower chances of developing cardiovascular mortality or risks associated with that according to multivariate-adjusted analyses. As the testosterone level was increased in the serum, the chances of developing cardiovascular events were decreased in men with the highest quartile containing the odds ratio of 0.53 (95% CI 0.32–0.86) compared with men providing lowest quartile. In the same way, increased level of testosterone lowers the all-cause mortality rate OR (0.59, 95% CI 0.42–0.85) for men in the highest quartile compared with men in the lowest quartile (Carrero et al. 2008). A Swedish study performed on 3014 men with a record of follow up for a mean time 4.5 years reported that men having lowest quartile value such as ($\leq$336 ng/dl) were at higher risk of about 65% of all-cause death as compared to men with 2–4 quartile (HR 1.65, 95% CI 1.29–2.12) (Tivesten et al. 2009).

On the other hand, a study conducted on 3690 men with a mean follow-up duration of 7.1 years documented a U-shaped relationship between endogenous testosterone and all-cause mortality. According to that, men reaching second and third quartile of testosterone level were at lower risk of mortality as compared to men at extreme values such as highest or lowest quartile level (Seftel 2014). On the other hand, some other reports contradicted to these findings and reported that there is no link between testosterone and the mortality (Shores et al. 2014). Another study participated 1804 men with a mean age of 50 years said that during 15 years of treatment the patients who died had come up with the lowest level of testosterone, however, this connection was not so valid as many risk factors were amended according to the patient's condition (Chan et al. 2016).

When serum T, cardiovascular events and all-cause mortality were analyzed under meta-analysis, it was found that testosterone could act as a preventative action (Araujo et al. 2011). It was also found that lower the testosterone higher the risks linked with cardiovascular events (1.25, 95% CI 0.97–1.60), and all-cause death (RR 1.35, 95% CI 1.13–1.62) (Araujo et al. 2011). Though there is a great variety of studies such as duration, timings, etc. and the factors of participants such as testosterone level, age, etc. so careful monitoring is needed to conclude the data.

An interpretation of the interplay between endogenous testosterone level and cardiovascular risks/mortality

On a precise note, it was stated that the population study showed a big clash between different studies conducted. Thus, it was concluded on the bases of a majority of cases

that lower levels of testosterone are found to be a risk factor of cardiovascular mortality and all-cause mortality. Nevertheless, the interpretation of the data should be managed carefully because population studies cannot establish causality or exclude reverse causality. Thus, it was concluded that this kind of association could not be considered as a fundamental association (Wu et al. 2008; Haring et al. 2013a, 2013b). Some researchers reported testosterone as an advantageous effect against cardiovascular risk events, and it was proved after studying men with prostate cancer and treated with androgen deprivation therapy, and it resulted in cardiovascular mortality and metabolic syndrome due to low level of testosterone (Alibhai et al. 2009; Azoulay et al. 2011; Martin-Merino et al. 2011; Hu et al. 2012; Keating et al. 2013). As in these cases, men are in the threshold.

Retrospective assessment of the influence of exogenous T

Since the past, it was found that testosterone therapy increases the risks of cardiovascular disease as reported by health care researches and the prescription database as discussed in Table 4. From a research study conducted on 8709 men who have gone through the process of coronary angiography and showed lower testosterone level viz. <300 ng/dl, via an investigation based on adjustment, it was found that 1223 men treated with testosterone replacement therapy had 29% chances of increased risk of cardiovascular disease such as stroke, death as compared to those men who did not take testosterone replacement therapy (Tan et al. 2015). Though, despite the diversity in the serum testosterone that was low in case if testosterone treatment was started viz. 175 ng/dl as compared to those who did not get through testosterone therapy viz. 205 ng/dl, the same study was performed without adjustments. When the patient's facts were taken into account, and the limitations of using this therapy were confronted, it was found that these data could not apply to the general population.

Furthermore, a large scale cohort study was performed on 55 593 men. In the context of insurance database, those men were receiving testosterone replacement therapy in patients with lower TST serum. As a result, it was concluded that the risk of myocardial infarction had been increased up to 36% within the 90 days of prescription. Thus, another factor like age had also been involved the risk such as for ≥65 years' men increased to about 119%, and for nearly 75 years of men it increased up to 243% (Page 2014). His study also elaborated that the non-fatal myocardial infarction was even common in youngsters who already had cardiovascular disease. On the other hand, the men who forget to fill their prescription again had lower the risk of cardiovascular disease within 91–180 days. Thus, it was suggested that high risk of cardiovascular disease was due to testosterone therapy.

An additional study regarding risk of testosterone therapy evaluated the fact that the higher occurrence of myocardial infarction was due to first time introduction of testosterone therapy after 90 days (RR 1.41, 95% CI 1.06–1.87), while the testosterone treatment in progression or testosterone treatment in the past did not connect with myocardial infarction (Etminan et al. 2015).

Another drawback linked to this study was that the low testosterone level was not set as criteria for the control

Table 4. Meta-analyses on the association between cardiovascular risk and TRT.

Reference	A sample size of studies included and age of subjects in years	Cardiovascular events/mortalities		Remarks
		Treatment	Placebo	
Tivesten et al. (2007)	A study conducted in 2016 encompassing 108 studies covering men having age <65 years.	E_{cv}/n_t 18/2037 E_{cv}/n_t 56/3476	E_{cv}/n_t 14/1331 E_{cv}/n_t 48/2713	No association between MACV and testosterone therapy (OR 0.97 CI_{95} 0.64–1.46)
Mäkinen et al. (2008)	A study conducted in 2016 encompassing 30 studies covering men having age 42–79 years.	E_{cv}/n_t 69/3230	E_{cv}/n_t 53/2221	No association of testosterone therapy with myocardial infarction (OR 0.87 CI_{95} 0.39–1.93) and mortality (OR 0.88, CI_{95} 0.63–7.54)
Page et al. (2008)	An analysis conducted for the coverage of 45 studies comprising men having age 56–72 years.	E_m/n_t 22/1497	E_m/n_t 22/1175	Testosterone therapy was not associated with cardiovascular risk (RR 1.10 CI_{95} 0.86–1.41). However, the therapy in patients with age ≥65 was associated with cardiovascular risk (RR 2.90 CI_{95} 1.35–6.21).
Sharma et al. (2016)	A study conducted in 2016 encompassing 27 studies covering men having age 24–87 years.	E_{cv}/n_t 115/1733	E_{cv}/n_t 65/1261	Testosterone therapy resulted into 54% increase in cardiovascular risk (OR 1.54 CI_{95} 1.09–2.18).
Haffner et al. (1993)	A study conducted to encompass 51 assessments covering men having age 18–88 years.	E_{cv}/n_t 66/1750	E_{cv}/n_t 43/1226	No significant association between cardiovascular risk and testosterone therapy.
Zhang et al. (2014)	A study conducted in 2014 encompassing 6 investigations covering men having age ≥40 years.	E_{cv}/n_t 14/161	E_{cv}/n_t 7/147	(OR = 1.82, CI_{95} 0.78–4.23), fatal to nonfatal MI ratio 2.24 (CI_{95} 0.50–10.0)
Yildirir et al. (2001)	An investigation conducted to encompass 19 studies covering men having age ≥45 years.	E_{cv}/n_t 18/651	E_{cv}/n_t 16/433	(OR = 1.14, CI_{95} 0.59–2.20)

MACV: major adverse cardiovascular events; n_s: a sample size of studies included; n_t: number of patients who received testosterone therapy; n_p: number of patients who received placebo; E_{cv}: number of cardiovascular events; E_m: events resulting into death; OR: odds ratio; RR: relative risk; CI_{95}: 95% confidence interval. Symbols used are: A: associated; CA: conditionally associated; NA: not associated.

group. However, another study documented that one testosterone injection on 6355 Medicare beneficiaries on ≥66 years aged men had the results just like 19 065 controlled men. It was stated that testosterone therapy is not responsible for increase induction of myocardial infarction (HR 0.84, 95% CI 0.69–1.02), yet it has found as beneficial results in men with a higher risk of myocardial infarction (HR 0.69, 95% CI 0.53–0.92) (Baillargeon et al. 2014). The same results were also discussed in RHYME cohort (Debruyne et al. 2017).

A study on the effects of testosterone therapy is performed on 10 311 men with mean persuasion time of 5.3 years reflected that this therapy was related to decreased mortality (HR 0.88, 95% CI 0.84–0.93) especially in men who used this therapy for long-duration while 28 029 men were selected as controlled (Wallis et al. 2016). Furthermore, the prolonged use of testosterone treatment therapy has shown lesser chances of inducing cardiovascular events in men as compared to controlled (HR 0.84, 95% CI 0.72–0.98). On the other hand, the men who received testosterone treatment therapy for a short duration of 60 days in comparison with controlled men (HR 1.26, 95% CI 1.09–1.46) (Wallis et al. 2016), approved the results of other studies (Page 2014; Etminan et al. 2015). The above studies documented that though the risks of cardiovascular disease have increased with the use of testosterone treatment therapy. Yet, the risk can be declined by using the medicine for a longer period.

However, the studies ex post facto did not turn up testosterone serum concentrations; as result acceptability of treatment, therapy might not be evaluated. But a research study gauged difference of cardiovascular effect on men who were prescribed with a therapeutic level of testosterone and men who remained on the minimal concentration of testosterone (Sharma et al. 2015). The research disclosed the fact that lower the levels of testosterone higher the risk of cardiovascular disease such men with consistently low levels of testosterone had enhanced risk of cardiovascular events in comparison to men who achieved a therapeutic level of testosterone (Sharma et al. 2015). Furthermore, the threat linked with cardiovascular actions such as stroke and myocardial infarction in men with continuous low levels of testosterone was the same as the men who were without any treatment and the reports related to that data had been long established in other research articles (Anderson et al. 2016; Oni et al. 2017).

The preparation of testosterone was known to have a strong impact on cardiovascular events (Layton et al. 2015). A trial conducted on 544 115 men less than 18 years of age who started IM injection of testosterone replacement therapy were at high risks related to heart and vessels that include unstable angina and stroke, hospitalizations, and death in men who were using transdermal testosterone gel (Layton et al. 2015). This research study was unable to describe treatment serum testosterone concentrations; however, the calculation of the concentration of on-treatment intramuscular testosterone level might be accessed via monitoring circulating concentration of testosterone.

In short, these studies included ex post facto, and the prescription database explicit all the essential information related to cardiovascular effect occurred due to testosterone replacement therapy (Table 4). As these retrospective studies have been come up with some limitations; thus, the cardiovascular safety cannot be documented with the help of these studies.

Randomized controlled trials for the assessment of the influence of exogenous testosterone

So far, no dummy run is available to assess differences in rates of cardiovascular events among individuals treated with testosterone replacement therapy with placebo. Additionally, a not standardized format has been followed in those cases where testosterone replacement therapy was not up to the mark (Basaria 2014). This segment of study will sum up the experimentation where cardiovascular events with atherosclerosis progression as the initial result were described.

Danish trials for the assessment of the influence of exogenous testosterone

In 1986, a Danish-based study was conducted on the men who survive after alcoholic cirrhosis and evaluated the role of testosterone replacement therapy and its results and resulted in the increased mortality. It was documented as one of the first studies to be reported the increased mortality and according to that trial 221 men were treated with a micronized dose of 600 mg of testosterone or either they remained placebo (Gluud and Henriksen 1987). But test trial was pre-paused because of 17% higher mortality rate in the testosterone group and comparison, with those who were inactive to the drug. Only one death was the result of a CVA (MI).

Assessment of the influence of exogenous testosterone through testosterone in older men (TOM) trials

In 2010, the summary of the results accumulated by performing trials on testosterone group versus placebo group was stopped before time by the Data and Safety Monitoring Board due to a higher rate of cardiovascular-related events (Hall 2010). Men with age below 65 years were covered in the test, which was having minimal or no testosterone along with the limited movement. The tested individuals received transdermal 100 mg of testosterone or placebo in gel form daily for at least six months. A total number of 23 men encountered with risks associated with a cardiovascular system according to the report and five men in the placebo group. The events came up with both results like atherosclerotic and non-atherosclerotic events and became perceptible in the starting weeks of the treatment. Cardiovascular events like MACE could be seen only in those individuals fall in the testosterone group, out of which two men suffered from MI, one stroke, and one death (Hall 2010). Several men were treated with testosterone and encountered cardiovascular events had high levels of testosterone than the men who had met any cardiovascular events. Another evaluation revealed that these events had linked with variation in free

testosterone reported to be found in serum (Basaria et al. 2013). It was observed that those who were included in the TOM trial (Hall 2010) had generality of higher comorbidities out of which 80% had hypertension, 50% were obese, 25% had diabetes mellitus, and remaining half had been suffered in cardiac disease. The rate of comorbidities is commonly high in men already reported with ill health (Newman et al. 2001, 2006).

Furthermore, the initial dose of testosterone gel chosen to start the trial was high as compared to that used in a clinical trial. But on treatment testosterone serum levels in persons included in TOM trial (Hall 2010) were same as it was in serum testosterone concentrations which were found in older men who participated in other trials and in whom there were slight chances of inducing cardiovascular events when treated with testosterone therapy. The varying nature of cardiovascular events found in the TOM trial indicated that the single methodology was not responsible for these events. The fast discrepancy of results between two groups also lead to the indication of some malicious processes may be the cause of these events which was later on confirmed by using different research studies (Page 2014; Etminan et al. 2015).

Assessment of the influence of exogenous testosterone through T's Effects on Atherosclerosis Progression in Aging Men trials

The T's Effects on Atherosclerosis Progression in Aging Men (TEAAM) trial documented the role of testosterone in the development of subclinical atherosclerosis in the common carotid artery and the coronary arteries (Rai and Ramasamy 2016). The total of 308 volunteers was included during the trial which was aged below 60 with 100–400 ng/dl total testosterone or more than 50 pg/ml free testosterone concentration in serum. The trial started by treating a patient with 75 mg of the gel form of testosterone or either placebo gel. Intercession of three years disclosed that testosterone treatment was not associated with the progression of carotid intima-media thickness (Rai and Ramasamy 2016). Furthermore, both of the groups did not show any variation in coronary artery calcium scores, which indicated that this calcified coronary plaque did not link with testosterone. Considering the slow progression of atherosclerosis, the period of 3 years may consider inadequate to determine differences of atherosclerosis progression in two groups.

Some of the individuals present in the trial came up with cardiovascular events, and MACE was the same in two groups. Thus, out of five men and all required coronary revascularization in one group while two men out of five required revascularization using a placebo. MI was seen in three men in the testosterone group and two men in the placebo group; three men encountered with stroke in the testosterone group versus none in the placebo group, and one participant died from a cardiovascular-related event in the testosterone group versus none in the placebo group (Rai and Ramasamy 2016).

Assessment of the influence of exogenous Testosterone through Testosterone Trials (TTrials)

A set of seven trials, which were highly coordinated, double-blind, placebo-controlled and were given the name of TTrials (Akishita et al. 2010; Snyder et al. 2016; Resnick et al. 2017; Roy et al. 2017), assessed the effects of testosterone replacement therapy on sexual, corporal, strength, anemia, bone mass, perception power, and coronary artery plaque volume (cardiovascular substudy). In the initial trial, 790 men aged below 65 years and testosterone level below 275 ng/dl and average serum were given a daily dose of 50 mg transdermal testosterone randomly or placebo gel for one year (Snyder et al. 2016). A total of 138 persons included in the TTrials underwent a CT angiography scan during cardiovascular substudy for assessment of non-calcified and calcified coronary artery plaque volume and coronary artery calcium score (Akishita et al. 2010). After the intervention of one year, it was disclosed that testosterone treatment was not responsible for the development of calcified plaque as compared to placebo. Still, there was a surprising increase in the volume of non-calcified plaque in comparison to the placebo group (Akishita et al. 2010). Still, the inferences of the trial were doubtful as there was no difference in cardiovascular events in two groups, and seven participants faced a MACE in each group (Snyder et al. 2016).

Assessment of the role of exogenous testosterone as a tool of contraception

In males, use testosterone therapy as contraceptive purposes. Its success has been assessed in many trials, although in many such trials doses of testosterone used caused supra-physiological serum testosterone concentrations, so it was needed in such trials to use treatments, which maintain a level of testosterone within the normal range of healthy young men (Handelsman et al. 1996; Paulsen et al. 1996; Meriggiola et al. 1997, 1998; Matsumoto et al. 1999; Wu et al. 1999; Zhang et al. 1999; Mulders et al. 2000; Kinniburgh et al. 2001; Anderson et al. 2002a, 2002b; Gonzalo et al. 2002; Kinniburgh et al. 2002; Morselli-Labate et al. 2002; Gu et al. 2003; Huebler et al. 2003; Matsumoto et al. 2003; Brady et al. 2004; Gu et al. 2004; Coviello et al. 2005; Meriggiola et al. 2005; Page et al. 2006; Qoubaitary et al. 2006; Wang et al. 2006, 2007; Gu et al. 2009; Mahabadi et al. 2010; Nieschlag et al. 2011; Ilani et al. 2012; Behre et al. 2016). Thus, doses were given via different ways, including intramuscular injections (Paulsen et al. 1996; Meriggiola et al. 1997, 1998; Matsumoto et al. 1999; Wu et al. 1999; Zhang et al. 1999; Mulders et al. 2000; Gonzalo et al. 2002; Morselli-Labate et al. 2002; Gu et al. 2003; Huebler et al. 2003; Matsumoto et al. 2003; Gu et al. 2004; Coviello et al. 2005; Meriggiola et al. 2005; Qoubaitary et al. 2006; Wang et al. 2007; Gu et al. 2009; Nieschlag et al. 2011; Behre et al. 2016), subcutaneous pellets (Handelsman et al. 1996; Kinniburgh et al. 2001; Anderson et al. 2002a, 2002b; Kinniburgh et al. 2002; Brady et al. 2004; Wang et al. 2006), transdermal gels and patches (Gonzalo et al. 2002; Page et al. 2006; Mahabadi et al. 2010; Ilani et al. 2012) with a different treatment period of 20 days

to 30 months. As these studies lacked the way to evaluate cardiovascular events, none of the MACE was noticed during treatment. A progestin was also given along with testosterone for the improvement of suppression of spermatogenesis, which by itself may affect the risk of cardiovascular disease. It was observed that in some studies that HDL-cholesterol concentration was reduced due to testosterone treatment (Gonzalo et al. 2002; Gu et al. 2003; Brady et al. 2004; Gu et al. 2004; Mahabadi et al. 2010).

Assessment of the role of exogenous testosterone based on a meta-analysis of randomized clinical trials

When published results of testosterone replacement therapy were reported via meta-analyses, controlled attempts have been there to explain the linkage between testosterone administration and cardiovascular events. However, these trials enrolled men who were having different characteristics, a difference of testosterone doses, formulations, and various treatment durations. Thus, most of such trials were not up to the mark to assess cardiovascular events; as a result, the conclusions of meta-analyses could not be harmonized.

Thus to prove this meta-analysis trial, cardiovascular events were assessed through randomized and controlled trials of testosterone therapy and was published in 2005 (Calof et al. 2005). The trial started with a sum of total of 19 trials, in which men inducted in the trial was $\geq$45 years' age having a low level of testosterone in the serum and treatment period selected was $\geq$90 days. Thus through this analysis, it was found that out of 651 men who were treated with testosterone replacement therapy, 18 men encountered cardiovascular events. In comparison, 16 men faced cardiovascular events out of 433 men who were using a placebo (Calof et al. 2005). It was found that cardiovascular event was not due to testosterone replacement therapy (OR 1.14, 95% CI 0.59–2.20). A meta-analysis was reverted for the young men. Another study evaluated the six trials and reported that out of 161 men in the testosterone group, 14 men were undergoing with a cardiovascular event of 147 men used a placebo, seven men were encountered with cardiovascular activity (OR 1.82, 95% CI 0.78–4.23) (Kelly and Jones 2014). The fatal and non-fatal odds ratio was found as 2.24 (95% CI 0.50–10.02). However, a more significant number of individuals was known to suffer from cardiovascular event receiving testosterone replacement therapy, yet this difference was not significant (Kelly and Jones 2014).

In 2010, a study was conducted using 51 randomized and non-randomized trials which added 2679 men. As a result, there was no effect of testosterone therapy seen to alter cardiovascular events and mortality (Fernández-Balsells et al. 2010). Though nine low or medium quality studies were performed to report the risk associated with cardiovascular events in men. In contrast to other studies, it was reported by meta-analysis trial that higher cardiovascular events were connected with testosterone therapy (Xu et al. 2013). This analysis utilized 27 trials with 2994 men in this study and reported 180 cardiovascular events. According to that, there was a 54% increase risk of cardiovascular events compared to other studies. The risk was comparatively high in those trials that were not aided by the pharmaceutical industry than to industry assisted study (OR 0.89, 95% CI 0.50–1.60) (Xu et al. 2013). In 2016, considering the age and testosterone formulation as the main factor, a total of 45 trials were performed and found no link between testosterone therapy and cardiovascular events compared to placebo (RR 1.10, 95% CI 0.86–1.41) (Albert and Morley 2016). Though the aged men of greater than 65 years were reported with higher cardiovascular events (RR 2.90, 95% CI 1.35–6.21), and it was significantly evident in the 1st year of treatment. However, it was seen that transdermal testosterone therapy has a higher incidence of cardiovascular events versus placebo (RR 2.80, 95% CI 1.38–5.68), on the other hand, testosterone administered intramuscularly did not show any cardiovascular risk (RR 0.96, 95% CI 0.46–1.98). The related observations were published in 2015 and contradict with the retrospective studies which reported IM administration of testosterone as a cardiovascular event as compared to gel form (Layton et al. 2015).

A total of 30 randomized trials reported under meta-analysis included 3230 men who were treated with testosterone therapy and out of which 69 men came up with cardiovascular events compared to 2221 men using placebo that underwent with 53 events (Xu et al. 2013). It was also reported that testosterone treatment was not responsible with myocardial infarction or death (OR 0.88, 95% CI 0.55–1.41). However, the risk of stroke was higher with the use of placebo, but it was not a noticeable difference (Xu et al. 2013). Another meta-analysis of 93 randomized trials in adults using testosterone replacement therapy had no effect on MACE than placebo (OR 0.97, 95% CI 0.64–1.46) (Corona et al. 2018). These meta-analyses were presented with low to medium quality; thus, none of these analyses had designed adequately to assess cardiovascular events.

Influence of testosterone therapy on the cardiovascular system

The population studies of various regions documented that men are more prone to cardiovascular risks than women (Tunstall-Pedoe et al. 1999; D'agostino et al. 2008; Kappert et al. 2012). Along with that sex steroids, to some extent, took part in increasing cardiovascular risk (Kalin and Zumoff 1990). From previous five years, a vast data have been built on molecular mechanisms of effect on cardiovascular system due to testosterone therapy (Figure 1) and also the discussion of modification of risk factors due to testosterone replacement therapy.

In vitro *and pre-clinical studies for assessing the effect of testosterone therapy on the cardiovascular system*

Various types of pre-clinical studies have been conducted to evaluate the effect of testosterone therapy on cardiovascular activity.

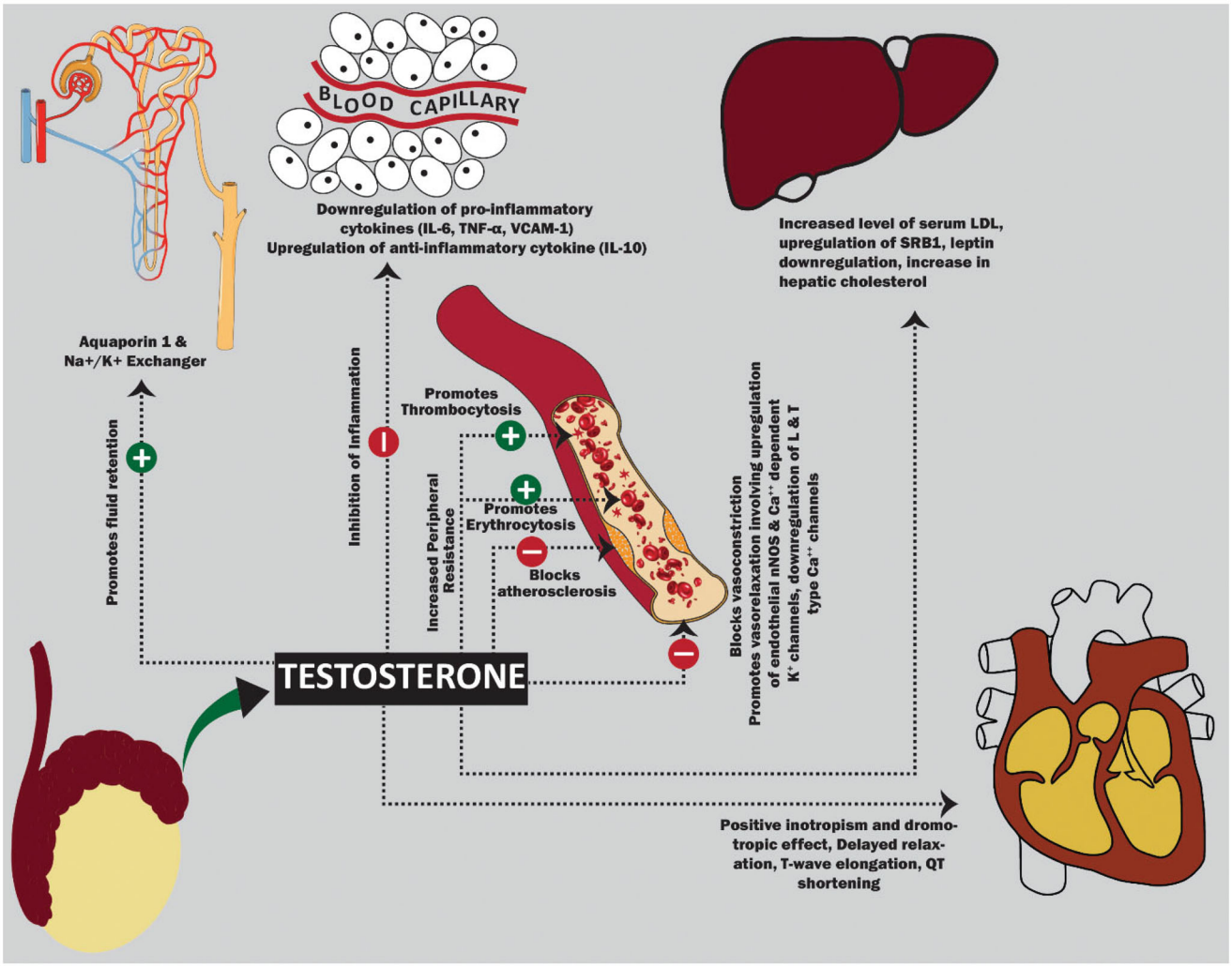

Figure 1. Physiological effects of testosterone on different organs and tissues. Testosterone is released from testis and to some extent from the adrenal medulla. Based on various pre-clinical and clinical studies, testosterone has shown significant vasorelaxant, anti-atherosclerotic, anti-hyperlipidemic, and anti-inflammatory actions. Testosterone therapy has exerted fluid retention, positive inotropic influence on heart, T-wave prolongation, and reduction in QT interval in electrocardiograph. nNOS: neuronal nitric oxide synthase; TNF-α: tumor necrosis factor-alpha; IL: interleukin; VCAM1: vascular cell adhesion molecule-1; ICAM-1: intercellular adhesion molecule-1; SCARB1: scavenger receptor class B type 1; LDL: low-density lipoprotein.

Effect of endogenous testosterone on atherosclerosis

There are numerous studies available that slow down the development of atheroma formation. When high cholesterol diet has been fed to castrated rabbits resulted in the speedy establishment of aortic atherosclerosis (Alexandersen et al. 1999; Qiu et al. 2010), which can be reverted by use of testosterone therapy (Larsen et al. 1993; Alexandersen et al. 1999; Li et al. 2008). In the same way, a testosterone metabolite viz. 5α-dihydrotestosterone (DHT) also tends to slow the development of atherosclerosis (Qiu et al. 2010); it means that estrogen aromatization is not mandatory for this effect. A mice that had developed testicular feminization, when gave cholesterol-rich diet for 28 weeks and testosterone replacement therapy after that resulted in decreased lipid deposition on the aortic root. This effect can be reverted to some extent by adding up aromatase inhibitor or an estrogen receptor-α antagonist (Kelly et al. 2013). Just like that, an apolipoprotein E- deficient mouse could speed up atherosclerosis by knocking out Ar (Bourghardt et al. 2010). Thus, these studies documented that testosterone can hinder atherogenesis alone without any help from estradiol. In contradiction to the above research it was mentioned that castrated Ldlr-knockout mice when treated with testosterone or estradiol resulted in degeneration of atheroma. Thus, the protective effect vanished immediately when an aromatase inhibitor was co-administered with estradiol which is now considered as an essential function of estradiol (Nathan et al. 2001). When these studies were reviewed jointly, it was documented that estrogen and androgen had played their roles separately and slow down the development of atherosclerosis. In another study, it was determined that mice with testicular feminization were given high cholesterol food when treated with testosterone decreased inflammation locally within fatty areas of the aortic root (Kelly et al. 2013).

The *in vitro* research studies are conducted on atherosclerosis, and a different result has been found. The effect of testosterone on male rabbits was evaluated after endothelial denudation where a neointimal progression of plaque

formation in a cultured segment of the aortic ring was hindered by the use of testosterone in comparison with untreated rings (Hanke et al. 2001). Another important activity of testosterone is the degradation of tumor necrosis factor (TNF) which was produced by activation of vascular cell adhesion protein 1 (VACM1) in human aortic endothelial cells (Hatakeyama et al. 2002) and human umbilical vein endothelial cells (HUVECs) (Mukherjee et al. 2002). Thus, endothelial cell increased the adherence of leukocytes to endothelial cells by increasing expression of VCAM1; this could be considered as a primary step for the formation of atheroma (Cybulsky and Gimbrone 1991; O'Brien et al. 1993; Cybulsky et al. 2001). On the other hand, DHT therapy of HUVECs has increased the activation of VCAM1, thus stimulated the adherence of endothelial cells to monocytes in the human body. Yet, both of these could be blocked by androgen receptor antagonist (Mccrohon et al. 1999). Thus, it was documented that aromatization of testosterone is an essential step to convert it into estradiol; thus, it is necessary to make it useful as protective agent mainly when expressed on adhesion molecule (Mukherjee et al. 2002). Furthermore, androgen receptor binding to testosterone and DHT might show the different receptor conformations that further conscript various co-factors thus, it results in distinctive response through cells.

Regarding the effects of androgens on aortic smooth muscles, a different type of findings has been achieved. Testosterone treatment that occurred in aortic smooth muscle cells of human can stop the vascular calcification, which is produced due to inorganic phosphates via androgen receptor-dependent transactivation of growth arrest-specific protein 6 (GAS6) (Son et al. 2010). GAS6 is considered as a vital monitor of arterial calcification (Son et al. 2006, 2007). On the other hand, the testosterone and DHT treatment therapy in mouse amplify the vascular calcification produced by inorganic phosphates. In contrast, *Ar*-knockout mouse showed 50% lesser calcification as compared to wild type mice (Zhu et al. 2016).

Effect of endogenous testosterone on lipid metabolism

The data compiled from preclinical trials proposed the effect of testosterone on the metabolism of lipid and cholesterol. Thus, testosterone treatment therapy has the capability of multiplying the dose-dependent scavenger receptor class B member 1 (SRB1) expression in HepG2 hepatocyte cell line. Scavenger receptor class B member 1 is a known protein that monitors the cholesterol uptake from the circulating HDL by the liver (Langer et al. 2002). A similar effect was observed by treatment with DHT on castrated, obese mice (Movérare-Skrtic et al. 2006). Furthermore, decreased in LDL and cholesterol 7α-hydroxylase activity was seen with DHT treatment therapy (Movérare-Skrtic et al. 2006). According to these observations, it was found that LDL and serum cholesterol was decreased by the use of testosterone replacement therapy by increasing uptake and accumulating cholesterol and repressing discharge of cholesterol.

Like many other functions, testosterone also affects the activity of hepatic lipase (an enzyme known for removal of phospholipids and triacylglycerols from lipoproteins). Experimentation was conducted on old eugonadal men to find out the effect of testosterone on hepatic lipase. As a result, men treated with 600 mg per week of testosterone for three weeks increase the activity of hepatic lipase by 66% and HDL cholesterol was decreased in comparison with placebo (Herbst et al. 2003). Hypogonadism patients showed high hepatic lipase activity and low HDL cholesterol concentration in comparison with baseline when treated with 250 mg of testosterone enanthate from 4 weeks to 12 weeks (Tan et al. 1997). The alteration in hepatic lipase activity by the use of testosterone replacement therapy could be considered as a vital mechanism for lowering HDL concentration in some trials. Though various studies examined the lowering of HDL concentration directly linked with cholesterol efflux from macrophage by use of testosterone (Khera et al. 2011), it was also reported that alteration in HDL concentration is independent to the response to androgen therapy (Rubinow et al. 2012, 2018).

Effect of endogenous testosterone on platelet role and blood thickness

T also played a fundamental role in controlling erythropoiesis in mammals (Gardner and Pringle 1961; Shahani et al. 2009; Bachman et al. 2014; Gagliano-Jucá et al. 2018a, 2018b). However, the main adverse effect associated with testosterone replacement therapy is erythrocytosis (Seftel 2019). This adverse effect is due to the high viscosity of blood in rabbits as well as in mice (Zhao et al. 2013; Guo et al. 2015) that can result in hindering the normal blood flow, which results in creating resistance. However, a higher number of red cells always have valuable effect as it raises the tendency of oxygen transport to its threshold level. At the same time, it vanished its beneficial effects when blood flow decreased to its lowest (Ahmadian et al. 2017). Besides, the fact was already documented that platelet aggregation and the hematocrit value are interrelated to each other and it resulted in the risk of thrombus formation due to increasing red blood cells concentration (Eugster and Reinhart 2005).

Similarly, testosterone replacement therapy enhances the aggregation ability of *ex vivo* platelets by increasing thromboxane receptor A2 density on human platelet cells (Ajayi et al. 1995). On the contrast, it was seen that platelet aggregation and thromboxane A2 receptor density were decreased in old castrated men versus uncastrated men (Ajayi and Halushka 2005). The report accumulated from these data suggested that testosterone can give a prothrombic effect.

Effect of endogenous testosterone on the role of the endothelium

Just like other studies, some studies suggest testosterone replacement therapy can act as a vasodilator by using several different mechanisms. When male rabbits were tested for endothelium, stripped coronary artery and aortas showed vasodilator effect; thus, it resulted in a fact that the function of vasodilatation is independent of the endothelium (Yue et al. 1995). This vasodilatation was inhibited by a nonspecific

inhibitor of potassium channels instead of inhibiting aromatase, nitric oxide synthase, and androgen receptor antagonist. Thus, the results found out that Ca sensitive potassium channels and voltage-sensitive potassium channels opening membrane is responsible for monitoring vasodilatation produced by testosterone (Yue et al. 1995). Therefore, it was found that testosterone, along with DHT, created relaxation in isolated coronary arteries of kid instead of depending endothelium, and it happened by the opening of Ca and voltage-activated potassium channels (Deenadayalu et al. 2001).

In contrast to the above studies, some studies found that nitric oxide and endothelium are responsible for the vasodilatation effect of androgen (Tep-Areenan et al. 2002). Similarly, when dogs were treated with testosterone intracoronary, it increases the coronary blood flow that can be decreased with pretreatment of NOS inhibitor (Chou et al. 1996). Vasodilatation was also seen by use of testosterone via neuronal NOS (Perusquía et al. 2015). When conscious rats were treated with blocked ganglia through intravenous T, it resulted in a hypotensive response that was dose-dependent; however, this response can be inverted by pretreatment with neuronal NOS inhibitor (Perusquía et al. 2015). The vasodilatation due to NO was observed in pig iliac, mesenteric and renal arteries (Molinari et al. 2002).

T aggregation results in inhibition of voltage-dependent L type calcium channel subunit α1C (Cav1.2) that was further expressed in HEK 293 human embryonic kidney cell by the same mechanism which dihydropyridine calcium channel blocker used for expression (Scragg et al. 2004). The calcium channel antagonism was later found independent of androgen receptor and vascular endothelium (Jones et al. 2004). When testosterone or DHT reached a certain level, it causes induction of NO synthesis in human aortic endothelial cells by using androgen-dependent receptor mechanism (Yu et al. 2010). However, this effect cannot be reversed by pretreatment with an aromatase inhibitor or by the introduction of RNA that targets ESR1 into eukaryotic cells (Yu et al. 2010; Campelo et al. 2012). Thus, the above studies documented that testosterone in human coronary artery affected involvement of calcium-activated potassium channel and also affected BKCa in endothelium-dependent vasodilatory effect (Ruamyod et al. 2017).

Effect of endogenous testosterone on electrolyte balance

Salt and water retention is the fundamental function of androgens. Androgen stimulates the proximal tubules of the rennin–angiotensin–aldosterone system when experimented on rats (Ellison et al. 1989; Quigley 2008), which further boost Na^+/H^+ exchanger (Mačković et al. 1986), and increase the renal mRNA and proteins of epithelial sodium channel subunits in the distal tubule and collecting duct (Loh et al. 2016). Furthermore, testosterone tends to increase the transcript and aquaporin one protein in rat's nephrons (Herak-Kramberger et al. 2015). Thus, the data explicit that water and salt retention can be secured by androgens that in further cause the expansion of extracellular volume.

Effect of endogenous testosterone on the electrophysiology of heart muscles

Sex steroid hormones affect cardiac electrophysiology in that way; it eliminates the sex-related variations in several considerations regarding electrocardiogram, specifically in ventricular repolarization (Bidoggia et al. 2000). The research conducted on ventricular cardiomyocytes of guinea pigs reported that acute testosterone treatment therapy directly affects the duration of an action potential by shortening its time; thus for that purpose, it uses the androgen receptor-mediated pathway. This pathway work by gradually activating delayed rectifier potassium currents (*I*Ks; an outward repolarizing current) and also by inhibition of inward L-type calcium current that causes depolarization (*I*CaL) (Bai et al. 2005). Though the Cav 1.2 density and *I*CaL current were reported to be increased by high dose treatment therapy of testosterone (via activating androgen receptors) (Er et al. 2007). Similarly, testosterone multiplies the human Ether-a-go-go-Related gene (hERG; also known as KCNH2) potassium channel current. Thus, androgen receptor activation resulted in stimulating delayed rectifier potassium current (*I*Kr; an outward repolarizing current) (Ridley et al. 2008). Thus, the data accumulated resulted in the fact that testosterone therapy can induce cardiomyocytes repolarization, which further caused decrease in the duration of an action potential.

Effect of endogenous testosterone on the contraction potential of heart

T affects several body functions, such as relaxation and cardiac contractility. The testosterone treatment experiment on cardiomyocytes (ventricular) of rats that ingested the dose from acute to high concentration (1 μmol/l) resulted in inducing cardiomyocytes relaxation (Golden et al. 2005). Furthermore, the experimental rats that underwent orchiectomy resulted in high-level shortening of decreased cardiomyocytes and showed deferred relaxation after two weeks versus control rats (Curl et al. 2009). The related results were also achieved when surgical removal of testicles was undergone before 16 weeks (Golden et al. 2003). However, it was the surprisingly distinctive fact that short-term testosterone deficiency in rat Langendorff-perfused hearts that also experienced with orchiectomy did not come up with alteration in left ventricular function (Hsu et al. 2015).

In comparison to the above statement, long-term withdrawal effect of testosterone significantly damages the cardiac contractility, and this action can be reverted by use of testosterone (Hsu et al. 2015). When the amount of testosterone reached a certain level, it can increase the inotropic effect on the heart and also myocardial relaxation by directly acting on the α1-adrenergic receptor and β1-adrenergic receptor via the androgen receptor (Hsu et al. 2015). Thus, it can be concluded that the cardiomyocytes contractility and relaxation produced by testosterone suggested being induced by monitoring calcium level in cardiomyocytes (Curl et al. 2009). Therefore, action was done by discharging calcium using ryanodine receptor and clearing calcium rapidly from the cytosol by increasing activity of endoplasmic reticulum calcium ATPase (SERCA) (Witayavanitkul et al. 2013; Hsu et al. 2015).

Clinical studies for assessing the effect of testosterone therapy on the cardiovascular system

Effect of endogenous testosterone on coronary arteries

The research studies on animals reported the observations that testosterone can produce vasodilatation. A study conducted on 50 men in 1977 with ST-segment depression toward its baseline were treated with either 200 mg per week IM testosterone cypionate or placebo for eight weeks thus concluded that testosterone treatment causes reduced ST-segment depression by 51% (Jaffe 1977). This study also reported that testosterone produced dilatation which resulted in reduced risk of ischemia. In the same manner, when 13 men with coronary artery disease were administered with intracoronary testosterone showed vasodilatation in results (Webb et al. 1999). Thus, another trial of 46 men with angina treated with 5 mg transdermal testosterone patch for 12 weeks concluded in improving time interval up to 1 mm ST-segment depression when working out for testing on the treadmill (Romanò 2001). Another placebo-controlled clinical trial was conducted in 15 men out of which seven IM testosterone undecanoate for one year resulted in useful efficacy on myocardial ischemia (Mathur et al. 2009). Thus, testosterone is known for the betterment of myocardial perfusion as well as ECG parameters. The randomized controlled trial was performed on 22 men with coronary artery disease and treated with oral administration of testosterone undecanoate for about eight weeks that concluded in increasing myocardial perfusion in the region where unobstructed coronary arteries were detected by MRI (Webb et al. 2008). The results obtained by these findings are the vaso-relaxant effect of testosterone that assists the observations the androgen treatment therapy in men with prostate cancer made large arteries more rigid (Smith et al. 2001; Dockery et al. 2009). Thus, the findings achieved have proved the beneficial effect of the coronary vasculature. However, a minimal number of men were involved in these trials, and even the routes utilized for testosterone administration were not the practical routes of clinical practice, hence these results must be interpreted with care.

Effect of endogenous testosterone on the retention of fluids

The clinical trials on humans have proved the fact that testosterone can retain water and salt and expansion of extracellular volume just like animals (Johannsson et al. 2005). While the limitation of volume overload was seen in healthy men presented with pressure natriuresis, eventually, the men presented with liver or cardiac disorder might not be able to expel water immediately thus resulted in fluid overload. The clinical trials performed on older men came up with the increased rate of congestive heart failure (Hall 2010; Basaria et al. 2013).

Effect of endogenous testosterone on the electrophysiology of heart muscles

The data from the animal study were harmonized with human studies about the efficacy of testosterone on cardiac electrophysiology. The newborn babies (males or females) have the same QT interval (Stramba-Badiale et al. 1995), but this similarity could be observed until 10 years of age (Alimurung et al. 1950). Though, reaching to the puberty, the QTc interval appears shorter in boys as compared to girls (Rautaharju et al. 1992; James 2011) that result in significant efficacy on the cardiovascular system due to testosterone therapy. In the National Health and Nutrition Examination survey, the 2942 men were tested for testosterone and found that testosterone level was negatively correlated with QTc interval (James 2011). In the same way, when ECGs were analyzed in 2755 men in Heath 2000 study it reported that men with greater level of testosterone reported with early repolarization and rapidly ascending ST segment versus men with minimal or no testosterone (Junttila et al. 2013). Sometimes, the QTc interval prolonged due to age factor as age caused decrease in serum level of testosterone (Vicente et al. 2014). The randomized trials on the community dwelling men and men with disease of chronic heart disease started secondary analyses which result in shortening of QTc interval (De Bruyne et al. 1999). The fact that the testosterone also affected the conducting system of heart was proved by the experimentation on androgen deficient men who were also suffered with prostate cancer that concluded the prolonged QTc interval (Gagliano-Jucá et al. 2018a, 2018b). As prolonged QTc interval is directly linked with risks related to heart such as tachyarrhythmia in ventricles and cardiac failure (Zhang et al. 2011; Noseworthy et al. 2012; Nielsen et al. 2014). Thus, it can be concluded that testosterone could be linked with arrhythmias. Thus, a similar kind of study was conducted in European pharmacovigilance database which reported that the hypogonadism in men was caused by the torsades de pointes, which can be reverted by use of testosterone therapy (Salem et al. 2018).

Effect of endogenous testosterone on the cardiac function

Women have strong circulatory system of heart as it contains high ejection fraction versus men (Buonanno et al. 1982), men can increase their ejection fraction of heart by exercise (Hanley et al. 1989; Merz et al. 1996). In this context, it was also seen that testosterone replacement therapy improves the aerobic capacity in aged men (Hayes et al. 2013; Traustadóttir et al. 2018). Some more studies also demonstrated the treatment efficacy of testosterone in those men who were diagnosed with congestive cardiac failure. To analyze the hemodynamic effect of testosterone in 12 men, a small trial was conducted in those hearts in which mild to severe left ventricular deformity was observed. Hence, for treatment purpose two days treatment therapy was performed with 60 mg of daily dose which resulted in recovery of cardiac output along with betterment of vascular resistance (Pugh et al. 2003). There are some more trials available about the chronic treatment of testosterone and their effect on cardiac function in men who suffered with congestive heart failure. During the study trial on 76 men showing mean ejection fraction of 32.5%, testosterone treatment with transdermal patch of 5 mg for 1 year showed direct effect and recovering functional capacity as compared to placebo

(Malkin et al. 2006). Similarly, out of 13 men from the group treated with testosterone showed betterment in one NYHA class in comparison with three men treated with placebo (214). The results associated with 24 weeks' testosterone undecanoate treatment of 1000 mg was confirmed by studying the case study on 70 aged men suffered with congestive heart failure (NYHA class II or III) and <40% ejection fraction compared with placebo (Malkin et al. 2006). Thus, it was found that testosterone treatment has the ability to enhance oxidative capacity when evaluated by walking for duration of total 6 min (Malkin et al. 2006), and also has the tendency to better the arterial baroreflex activity which is normally decreased in CHF patients (Mortara et al. 1997).

Effect of endogenous testosterone on atherosclerosis

Epidemiological studies summed up the link between testosterone levels versus atherosclerosis occurring in men. The Tromso study performed on 1482 men with age from 25 to 84 years evaluated that the decreased testosterone was not directly linked with carotid artery intima-media thickness, even after modifying two factors like age and cardiovascular risks (Svartberg et al. 2006). When 1101 men were persuaded for about 7 years, it was showed that there was no link among testosterone and development of carotid intima media thickness (Vikan et al. 2009). The research study performed on 196 aged men for duration of 4 years when studied on smaller levels documented that development of intima media thickness was linked inversely with the testosterone free concentration (Muller et al. 2004). This opposite relation among testosterone versus development of atherosclerosis seemed to be stronger with low level inflammation (220). Some of these studies also assessed the relation among aortic and coronary atherosclerosis and the testosterone concentration (Yildirir et al. 2001; Li et al. 2012; Park et al. 2012; Khazai et al. 2016; Travison et al. 2016). A small study of 90 men came up with coronary artery disease conducted experiment for the evaluation of angiography among low bioavailable testosterone compared with healthy men even after monitoring major factors of age and BMI (Yildirir et al. 2001). The bioavailable testosterone concentration was connected oppositely with the coronary calcium score among 105 Korean men who were non-obese and middle age (Park et al. 2012). A study performed on total of 803 men with elective angiography having low level of testosterone showed more severe symptoms of coronary artery disease (Li et al. 2012). An opposite relation was seen from the Offspring and Third Generation cohorts of the Framingham Heart Study among testosterone versus coronary artery calcification in 1654 men, though the connection between them was no longer evident after modification of factors associated with it (Travison et al. 2016). An opposite link has also been documented among testosterone concentration and peripheral artery disease (Travison et al. 2016). The results summed up by the above trials were opposite from the trials that found out that randomized controlled trials on development of atherosclerosis versus testosterone is independent of testosterone concentration. In TEAAM trial, it was reported that testosterone treatment therapy was deprived of any effects on alteration on carotid intima media thickness or coronary calcium stores (Rai and Ramasamy 2016). Furthermore, in another TTrials concerned with cardiovascular activity, the level of noncalcified plaque was higher in those men treated with 1-year treatment therapy of testosterone versus placebo (Akishita et al. 2010). The contradictory results achieved by epidemiological studies and data published via clinical trials among testosterone therapy and development of atherosclerosis make it mandatory to analyze cardiovascular activity deliberately (Akishita et al. 2010).

Effect of endogenous testosterone on lipid metabolism

From the lipo profile data study, it was found that the level of testosterone is inversely connected with lipoprotein profile data (Haffner et al. 1993; Mäkinen et al. 2008; Zhang et al. 2014). There are several studies present that documented the decreased amount of HDL cholesterol (Haffner et al. 1993; Mäkinen et al. 2008; Zhang et al. 2014). The efficacy of testosterone on circulating lipids always remains incoherent in the trials. When randomized trials were conducted on 108 aged men with transdermal testosterone treatment for three years, it resulted in no effect on lipoproteins (Snyder et al. 2001). The same results achieved in TEAAM trial (Rai and Ramasamy 2016) that conducted meta-analyses concluded with the decreased amount of total cholesterol and LDL cholesterol and a small decrease in HDL-cholesterol while the triglyceride remains unchanged when 19 study trials were done to find out the effect of testosterone therapy on 272 men (Whitsel et al. 2001). Same were the results of TOM (Hall 2010) and TTrials (Le et al. 2018a, 2018b) which showed the linkage of testosterone treatment with reduction of bad and good lipoproteins levels, however to the extent of cardiovascular disease risk, the role of these changes was still a dilemma. There was no apparent sufficient effect of testosterone on HDL function (Rubinow et al. 2018). The levels of lipoprotein decreased up to 21% were observed from baseline during a trial in men suffering from type 2 diabetes or metabolic syndrome and total testosterone level above 317 ng/dl with metabolic syndrome taking testosterone therapy for 6 months in comparison with those who were given placebo (Jones et al. 2011); however, the linkage between them was not clear.

Effect of endogenous testosterone on glucose metabolism

There is a permanent relationship among higher levels of testosterone and reduced threat of metabolic syndrome (Muller et al. 2005; Ding et al. 2006; Hong et al. 2013) and diabetes (Selvin et al. 2007; Yeap et al. 2009; Vikan et al. 2010), further, sharp extraction of replacement therapy of testosterone reduces insulin sensitivity in men with hypogonadism (Yialamas et al. 2007). Likewise, there is an increased risk of progression of metabolic syndrome and diabetes in those men whose prostate cancer was treated by androgen deprivation therapy (Smith et al. 2001; Basaria et al. 2006; Braga-Basaria et al. 2006; Shahani et al. 2008; Alibhai et al. 2009; Keating et al. 2013; Tsai et al. 2015; Le et al. 2018a, 2018b).

The results lead that testosterone stimulates sensitivity of insulin in men.

During autonomous research study, it was observed that linkage of serum testosterone levels and threat of inducing diabetes is not dependent on adiposity which means that risk factor for diabetes could be a testosterone level (Selvin et al. 2007; Yeap et al. 2009). On a large scale potential study, dependence on waist circumference (Vikan et al. 2010) of the threat of occurrence of diabetes with total testosterone in two lower quartiles leads that role of testosterone may be mediated by its effects on obesity and these observations were proved in another study (Hsu et al. 2014).

The risk of induction of metabolic syndrome (OR 1.64, 95% CI 1.41–1.90) in men with age ranging from 49 to 79 years (Huhtaniemi et al. 2015) with decreased amount of testosterone was more as compared to those with testosterone present in normal range and the adjustment of some factors such as lifestyle could not lower such risk (Huhtaniemi et al. 2015). No linkage was found between total testosterone or no testosterone and insulin resistance or threat of occurrence of diabetes in another study (Joyce et al. 2016).

There was no consistency between the result of random trials. By hyperinsulinemic–euglycemic clamp method (Kaplan et al. 2006; Dhindsa et al. 2016), an improvement in insulin sensitivity with testosterone therapy was observed but the results of randomized trials were conflicting some of which showed beneficial effects on insulin resistance (Shores et al. 2006; Jones et al. 2011) of testosterone therapy while others no effect (Gianatti et al. 2014; Huang et al. 2019), in short, on glycemic parameters effect of testosterone replacement therapy remained under doubt. Briefly, the imperative shortcomings in the study design, selection criteria and the analyses of most of such clinical trials and epidemiological studies may account for such conflicting findings.

Effect of endogenous testosterone on inflammation

There are many risk factors associated with cardiovascular disease that include inflammation (Saijo et al. 2009; Ruparelia et al. 2017). Several pro-inflammatory markers such as CRP and IL-6 can be used as predictive factors for the cardiovascular risk factors (Ridker 2003). In addition, some asymptomatic men with decreased level of TNF are also linked with clinical or sub clinical cardiovascular disease (Biasucci et al. 1999; Putko et al. 2014). There are several reported studies that contradict the connection between testosterone concentration and inflammatory markers (Pastuszak et al. 2017), while some other studies also came up with inverse relationship between testosterone and IL-6 (Pastuszak et al. 2017) and CRP (Störk et al. 2008). Still some studies contradict these findings (Kaplan et al. 2010). When small level of randomized controlled cross-over trial was conducted to find out the effect of testosterone replacement therapy on pro-inflammatory cytokines TNF and 1L-1β and inflammatory cytokines resulted in decreased TNF and IL-1b and increased anti-inflammatory cytokines, IL-10 (Maggio et al. 2006; Haring et al. 2012; Tsilidis et al. 2013). These findings concluded that testosterone treatment therapy could act as anti-inflammatory agent; however, these findings are contradicting to TTrial (Whitsel et al. 2001).

Effect of endogenous testosterone on the cardiovascular events – The TRNASVERSE trial

TRAVERSE trial (US National Library of Medicine) is known as the first randomized controlled trial that is used to analyze the cardiovascular events occurred due to testosterone replacement therapy. Thus, to start these trials in 2018 researchers plan a scheme with 6000 men ranging between 45 and 80 years of age who were at risk of cardiovascular disease were treated with testosterone <300 ng/dl in gel form or treated with placebo. The treatment period selected for this plan was 5 years and the foremost endpoint selected for this study was MACE (nonfatal MI, nonfatal stroke or death from cardiovascular causes). The other secondary results are achieved from occurrence of cardiovascular end point. The findings associated with these trials provide the documented results of cardiovascular safety by use of testosterone replacement therapy.

Conclusions

T plays an important role in regulating body functions which include cardiovascular system and metabolic system of the body. However, the population study contradicts the collected data and the clinical trials conducted yet. There are many studies still present that corroborate the facts about mortality due to testosterone replacement therapy. While several other studies in randomized trials documented the increased severity of cardiovascular risks due to testosterone therapy. Some contradictions were also seen in meta-analyses and were constrained in the range of low to medium trial. In addition, till now there is no authentic publication or research available that has the capability to evaluate its effect on cardiovascular risks associated with T. Thus for this purpose, a TRAVERSE trial (US National Library of Medicine) uses long-term treatment and the conclusions for the evaluation of cardiovascular events produced due to testosterone therapy. Thus, it is necessary to make patients aware about the cardiovascular events induced by testosterone replacement therapy.

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Declaration of interest

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