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Review

Testosterone therapy in hypogonadal patients and the associated risks of cardiovascular events

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ABSTRACT

Since the male secondary sex characters, libido and fertility are attributed to their major androgen hormone testosterone, the sub-optimum levels of testosterone in young adults may cause infertility and irregularities in their sexual behaviour. Such deficiency is often secondary to maladies involving testes, pituitary or hypothalamus that could be treated with an administration of exogenous testosterone. In the last few decades, the number of testosterone prescriptions has markedly increased to treat sub-optimal serum levels even though its administration in such conditions is not yet approved. On account of its associated cardiovascular hazards, the food and drug authority in the United States has issued safety alerts on testosterone replacement therapy (TRT). Owing to a great degree of conflict among their findings, the published clinical trials seem struggling in presenting a decisive opinion on the matter. Hence, the clinicians remain uncertain about the possible cardiovascular adversities while prescribing TRT in hypogonadal men. The uncertainty escalates even further while prescribing such therapy in older men with a previous history of cardiovascular ailments. In the current review, we analysed the pre-clinical and clinical studies to evaluate the physiological impact of testosterone on cardiovascular and related parameters. We have enlisted studies on the association of cardiovascular health and endogenous testosterone levels with a comprehensive analysis of epidemiological studies, clinical trials, and meta-analyses on the cardiovascular risk of TRT. The review is aimed to assist clinicians in making smart decisions regarding TRT in their patients.

1. Introduction

The secondary sex character, fertility and sexual desire in males are attributed to testosterone (testosterone) that is the major androgen hormone in humans. Sub-optimal testosterone levels may cause hypogonadism that could either be a result of the testicular malady (primary hypogonadism) or it could be secondary to a pituitary or hypothalamic insufficiency (secondary hypogonadism) [1]. However, a decline in serum testosterone levels is not always associated with testicular, pituitary or hypothalamic malfunctioning [2,3]. Such a decline in endogenous testosterone levels is quite obvious with increasing age, particularly after 50 years [4,5]. For instance, the testosterone levels are reported to decline by up to 49 % in patients from 50 to 80 years of age [2]. The clinical success and safety of testosterone replacement therapy (TRT) in young, as well as older men with androgen deficiencies, is wellestablished [6]. Interestingly, the endogenous testosterone levels in most of the men are found very closer to the lower physiological range of testosterone with as lower as 2% prevalence of symptomatic hypogonadism [7]. Hence, the safety of exogenously administering testosterone in such patients has always been a concern among clinicians, particularly in elderly patients who may have cardiovascular co-morbidities.

Besides its moderate benefits to treat hypogonadism, TRT is associated mainly as a marketing strategy to any single ailment that could be secondary to the lower testosterone levels in males [8]. A recent clinical trial has shown a moderate impact of testosterone therapy on sexual function and libido in elderly patients with no improvement in their vitality and physiological fitness [9]. Yet, the number of

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testosterone prescriptions in patients with sub-optimal testosterone levels has been tremendously increased worldwide [10–13]. Considering this, the food and drug administration (FDA) in the United States has issued alerts on testosterone prescribing in 2014 with serious concerns regarding its safety in elderly patients about its cardiovascular adversities [14]. The relationship between serum testosterone levels and cardiovascular morbidities has raised controversies. For instance, the international guidelines [15,16] as well as several cohort studies [17–23] have claimed an association of cardiovascular problems with lower serum testosterone levels. Yet, some retrospective studies have reported such cardiovascular adverse effects in men receiving TRT [23–26]. Albeit the cardiotoxic potential of TRT is well documented [27,28], a lot more reports have either claimed no relation or a positive impact of testosterone therapy on cardiovascular health [29–40]. Testosterone could be taken as a biomarker of health since a decline in serum testosterone levels is associated with an unhealthy lifestyle. Hence, a decline in its levels in patients with cardiovascular morbidities could be a result of physiological adjustments instead of being a cause of these cardiovascular ailments. Furthermore, the population data could not be used to determine the causality or reverse causality unless a molecular mechanism is established to prove such a correlation between serum androgens and cardiovascular ailments. Nevertheless, the prevalence of cardiovascular adverse effects in patients receiving testosterone therapy has raised a serious question among the clinicians regarding its safety.

To the best of our knowledge, no clinical trials have been published so far as to claim the safety of TRT. Although one such clinical trial to evaluate the cardiovascular toxicity of transdermal testosterone gel in hypogonadal men is currently in the process [41], the findings of this trial may take a decade to get published. Meanwhile, the present review may provide help to the clinicians regarding the available data of cardiovascular atrocities in men receiving testosterone exogenously. Thence, the present review has discussed epidemiological reports, retrospective studies, cohort studies and randomized control trials, meta-analyses to affirm association between endogenous testosterone levels, exogenous TRT and cardiovascular health.

2. Physiological significance of testosterone

As the major sex hormone, testosterone plays a significantly important physiological role in secondary sex characteristics, sexual behaviour, vigour, libido as well as the masculine buildup of skeletal musculature [42]. However, some studies have related the cardiovascular risk to male sex hormones [43] on account of a higher prevalence of cardiovascular ailments in men as compared to women [44–46]. Till today, a huge amount of data has been generated in pre-clinical and clinical studies. The findings of these preclinical and clinical studies are briefly narrated in the following section.

2.1. Pre-clinical evaluation

Being the major androgen hormone in men, testosterone may impart various effects on the cardiovascular parameters. A large number of studies have reported the potential impact of testosterone on the cardiovascular event by using animal models and cell culture techniques. Some of the most important findings on the subjects are reported below:

2.1.1. Vasorelaxation

Testosterone exerts a vasorelaxant effect by relaxing the vascular smooth muscles as evident from different *in vivo* and *ex-vivo* experiments. Hence, it plays a significant physiological role in modulating the overall blood pressure in men. The vasorelaxant effect of testosterone in different animal models and isolated tissue preparations was equally attested by the parallel *in vitro* studies in human and rat cell lines. The intra-arterial administration of testosterone exhibited potent vasodilatory effect in the coronary, iliac, renal and mesenteric arteries of pigs [47]. Initially, it was reported that this vasodilatory effect of testosterone did not involve endothelium, because of the fact that the testosterone-induced vasodilation was not attenuated by blocking endothelial mediators such as aromatase, nitric oxide synthase and androgen receptors in endothelium-denuded rabbit [48] and pig isolated coronary arteries [49]. Instead, the vasodilation was reversed by blocking voltage-gated and calcium-dependent potassium channels on the vascular smooth muscle cells [48,49]. As testosterone in physiological concentrations inhibited L-type and T-type calcium channels in A7R5 and HEK 293 cell lines, it was suggested that testosterone blocks the calcium influx through these channels to produce its vasorelaxant effect [50]. Such blockage of calcium influx by testosterone was not affected in endothelium-denuded isolated coronary arteries, suggesting no involvement of endothelium in testosterone induced inhibition of calcium influx [51].

Contrarily, the testosterone administration failed to induce vasorelaxation in dogs that were pre-treated with non-specific inhibitors of nitric oxide synthase [52]. The involvement of endothelial pathways was further suggested by another study which reported a significant decline in testosterone-induced vasorelaxation by blocking nitric oxide synthase or by removing endothelium [53]. Interestingly, the endothelial cells do express neuronal nitric oxide synthase [54], a blockage of which has reported to reverse testosterone-induced vasorelaxation in rats [55]. This endothelium-derived vasorelaxation resulted in an increase in nitric oxide synthesis in endothelial cells via androgen receptors [56,57]. It is recently reported that testosterone induces hyperpolarisation in human endothelial cells via calcium-dependent potassium channels (both SK and BK_{ca}) that may account for the endothelial-derived vasorelaxant effect of testosterone [58].

It is worth mentioning here that the vasorelaxant effect of testosterone does not affect blood pressure because such hypotensive effects of testosterone are countered by parallel retention of fluid volume. Testosterone induces reabsorption of water from nephrons via upregulating aquaporin 1 expression in rat nephron [59] stimulating renin-angiotensin-aldosterone system in rat proximal tubules [60,61], enhancing the expression of sodium proton exchanger [62], ing epithelial sodium channels in rat distal convoluted tubules and collecting ducts [63].

2.1.2. Prothrombotic effect

Testosterone treatment has resulted in an increased density of thromboxane A_2 receptor with a resultant boost in *ex vivo* platelet aggregation [64]. This finding was further confirmed by a subsequent study that compared the plasma testosterone levels, thromboxane A_2 receptor density and platelet aggregation in men who underwent orchiectomy [65]. With a significant decline in serum testosterone levels in castrated men, a fall in platelet aggregation and thromboxane A_2 receptor density was observed [66]. The cell membrane of platelets and megakaryocytes contain estrogen as well as testosterone-regulated androgen receptors that account for a boost in platelet aggregation in testosterone-treated cells [67]. Moreover, the expression of androgen receptors is upregulated by lower testosterone concentrations (1, 5 and 10 nmol/L) and downregulated at higher concentrations of 100 nmol/L [67]. Testosterone also stimulates erythropoiesis [68–71] that results into a excess production of erythrocytes in the blood of testosterone-treated patients [6]. In one way, such testosteroneinduced erythrocytosis enhances the oxygenating capacity of blood in athletes to withstand strenuous exercise, it also increases blood viscosity and peripheral resistance [72,73] that may precipitate into hypertension. Interestingly, this testosteroneinduced erythropoiesis may be countered by an increased destruction of erythrocytes in the capillary bed of contracting muscles during exercise [74]. Hence, testosterone-induced peripheral resistance and blood pressure may not produce that much significant clinical impact in athletes as it may exhibit in non-exercising older men receiving testosterone therapy. Moreover, the risk of thrombosis and coronary embolism is also directly linked with an

increasing number of circulating red blood cells [75].

2.1.3. Inotropic effect

The cardiomyocytes express androgen receptors that are sensitive to. When the isolated cardiomyocytes from rat ventricles were treated with a higher testosterone concentration of 1 μM for 24 h, the peak shortening (contraction) of myocytes was increased up to 21 % with 18 % decrease ($p < 0.02$) in time to get maximum peak contraction [76]. Further, testosterone treatment also resulted in a quick relaxation of the cardiomyocytes (up to 18 %, $p < 0.002$) after a stronger contraction. These findings suggested a positive ionotropic effect of testosterone on cardiomyocytes. A chronic testosterone-treatment of cardiomyocytes enhanced calcium influx in cardiomyocytes through L-type calcium channels ($I_{ca,\ L}$) that may account for this hypercontractility [77]. However, acute treatment produces an opposite impact on cardiomyocytes by directly blocking the L-type calcium channels. The enhanced $I_{ca,\ L}$ may account for an increase in peak shortening of cardiomyocytes as suggested by Golden and colleagues [76]. Likewise, a subsequent study claimed that the time to achieve maximum contraction was significantly increased *in vivo* in castrated rats [78]. Orchiectomy also resulted in a delayed relaxation; both of these findings were in close commitment with the previous reports [76,79]. Further, this delay in achieving peak contraction was attenuated after TRT [78,79]. The impact of castration on cardiac contractility did not affect the ventricular contraction up to a significant level up to 9 weeks of castration. However, a significant decline in ventricular impairment was observed following 16 weeks of castration [80]. At physiological concentrations, testosterone potentiated the adrenoceptor-mediated inotropic and lusitropic effect via modulating intracellular calcium ion levels through ryanodine receptors, sodium-calcium exchanger and sarcoplasmic reticulum calcium ATPase [81,82].

2.1.4. Elevation of t wave

One of the major differences between the male and female electrocardiograph (ECG) includes a more prominent T wave in males that almost equals the height of the R wave. This T wave is also prominent in females. However, its height is relatively lesser. Moreover, estrogen causes a relatively prolonged QT in women. These differences are attributed to the three major sex hormones i.e., testosterone, estrogen, and progesterone. QT interval indicates ventricular contraction whereas wave indicates ventricular relaxation. Acute exposure of guinea pig ventricular muscle cells to testosterone has resulted in a prolonged repolarisation phase without involving transcriptional changes. Such delay in cardiomyocytes was found to involve the activation of delayed potassium outward current from the treated cells and deactivation of L-type calcium channels [83]. Later, it was also revealed that the acute treatment of these cells with testosterone has also resulted in the induction of an hERG that stimulates the delayed potassium efflux from the cell membrane, causing the prolongation of T wave [84].

2.1.5. Antihyperlipidemic effect

As evident from both pre-clinical and clinical studies, the serum testosterone levels play a significant role in regulating blood cholesterol levels and hepatic cholesterol metabolism. Here we briefly discuss the pre-clinical studies that affirm a regulatory effect of testosterone on lipid metabolism. In simple words, the low-density lipoproteins (LDL) carry the cholesterol from the liver to blood vessels. In contrast, the high-density lipoproteins (HDL) assist cholesterol movement in the opposite direction, i.e., from blood vessels back to the liver. Hence the higher blood HDH levels are believed to be a sign of good cardiovascular health whereas the elevated LDL levels are directly associated with a higher risk of atherosclerosis. To induce hepatic absorption of cholesterol, HDL bind with scavenger receptor class B type 1 (SCARB1) receptors on hepatocytes. Hence, increased expression of SCARB1 on hepatocytes leads to greater absorption of cholesterol into the hepatocytes. Langer and colleagues reported a significantly higher *in vitro* expression of SCARB1 receptors on HepG2 cells after being treated with testosterone [85]. Their findings suggested that testosterone enhances the HDL mediated absorption of cholesterol into hepatocytes by upregulating SCARB1, marking testosterone as a sign of good cardiovascular health. Similar effect was observed in a subsequent *in vivo* study where higher expression of HDL was observed is dihydrotestosterone (DHT) treated castrated mice [86]. DHT also inhibited bile synthesis by suppressing cholesterol 7-α hydroxylase gene expression, thus reducing the excretion of cholesterol into bile [86].

2.1.6. Effect on atherosclerosis

Alexandersen and co-workers reported that the progression of arterial atheroma was significantly increased in castrated rabbits when switched on to a high-fat diet [87,88]. Moreover, this increased progression was markedly halted on TRT, suggesting a protective role of testosterone against atherosclerosis [87,88]. These results were in close commitment with a previous study by Larsen and colleagues [89] and were further affirmed by a subsequent work of Li and team [90]. Further, testosterone-therapy in obese mice with testicular feminisation also inhibited atherosclerosis induced local inflammation [91]. As testosterone is endogenously converted into estradiol by aromatase, it was later found that the inhibition of atherosclerosis is contributed by both testosterone and estradiol. Similar experiments were carried out by a nonaromatizable 5α-dihydrotestosterone (DHT) instead of testosterone [87]. As DHT also exhibited significant anti-atherosclerotic effect, the effect was mainly attributed to testosterone by involving a mechanism that seemed independent of its aromatization [87]. However, the studies have proven that is a suppression of atherosclerosis is contributed by both testosterone and estradiol. For instance, the administration of an aromatase blocker or estrogen receptor antagonist into the obese mice who underwent testicular feminization resulted in a partial blockage of the testosterone-induced suppression of aortic fat deposition [92]. Similarly, the testosterone and estradiol-induced anti-atherosclerotic effect were attenuated by administration of aromatase inhibitor in castrated LDL receptor knockout mice, confirming a considerable role of estradiol in preventing atherosclerosis [93].

In an *ex vivo* study on rabbit aortic denuded rings, fat deposition in tunica intima was significantly inhibited in the testosterone-treated group compared with control [94]. Considering the involvement TNF-α induced VCAM-1 expression in initiating atheroma [95–97], down-regulation of both TNF-α and VCAM-1 in testosterone-treated endothelial cells may contribute to its anti-atherosclerotic effect [98,99]. Contrarily, DHT exhibited a stimulatory effect on VCAM-1 in endothelial cells, an effect that was attenuated by blocking androgen receptor signaling [100]. Taking these results together into account, it might be concluded that the inhibitory effect of testosterone on VCAM-1 depends upon aromatase catalysed conversion of testosterone into estradiol [99]. In addition to its inhibitory effect on vascular fat deposition, testosterone blocks phosphate induced calcification of blood vessels via growth arrest-specific protein 6 pathway [101].

2.2. Clinical studies

The effect of endogenous testosterone levels/testosterone therapy on different physiological parameters is depicted in Fig. 1. The clinical studies on the physiological properties of testosterone are briefly summarised below.

2.2.1. Vasorelaxant effect

The clinical studies on the vasorelaxant evaluation of testosterone have published similar results that are in close agreement with the above described pre-clinical experiment. For instance, Jaffe and colleagues reported a 51 % improvement in the ECG of testosterone-treated patients (200 mg i/m per week for 8 weeks) who had a pre-treatment depression in ST-segment [102]. As the ST-segment

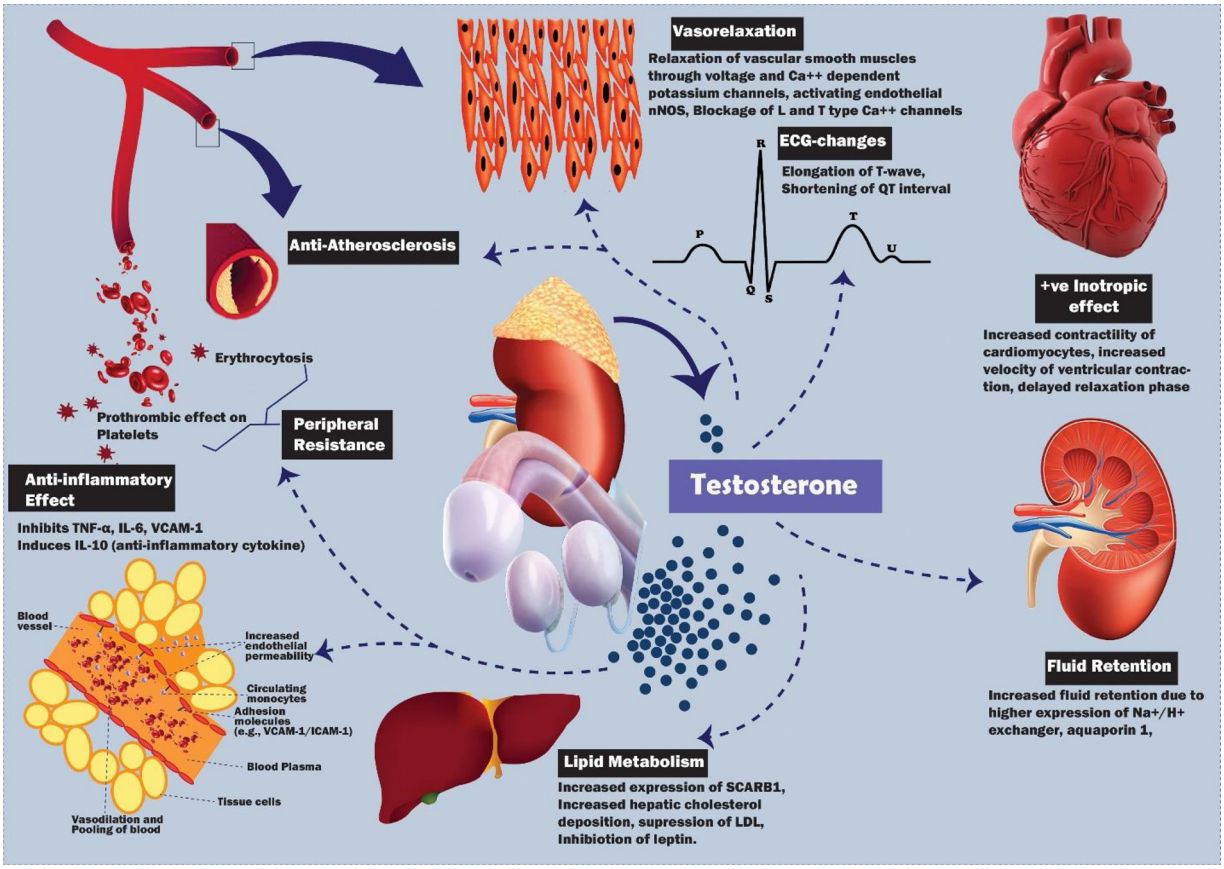

Fig. 1. Physiological effects of testosterone on different organs and tissues. Testosterone is released from testis and to some extent from the adrenal medulla. Based on several pre-clinical and clinical studies, testosterone has exhibited considerable vasorelaxant, anti-atherosclerotic, anti-hyperlipidemic and anti-inflammatory effect. Testosterone treatment has shown fluid retention, +ve inotropic effect on heart, T-wave prolongation and shortening of QT interval in electrocardiograph. Abbreviations: nNOS: neuronal nitric oxide synthase; TNF-α: tumour 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.

depression is always secondary to coronary ischemia, an improvement in such defects results from coronary vasorelaxation. In another study, the transdermal administration of 5 mg testosterone for 12 weeks significantly reduced the ST-segment depression up to 1 mm [103]. The vasorelaxant effect of testosterone was affirmed by two small clinical studies where an intracoronary [104] and intramuscular [105] administration of high testosterone doses resulted in coronary vasorelaxation. In addition, oral testosterone therapy in coronary heart patients for 8 weeks exhibited a moderate improvement in blood perfusion by exerting vasorelaxation in un-obstructed coronary arteries [106]. The vasorelaxant effect of testosterone is also evident from a significant arterial stiffness in prostate cancer patients who received androgen deprivation therapy [107,108].

2.2.2. Anti-atherosclerotic effect

In addition to the vasorelaxant effect of TRT in hypogonadal patients, there are various studies that have inversely correlated serum testosterone levels with the incidence of atherosclerosis. For instance, the low serum testosterone levels were found inversely related to the progression of carotid atherosclerosis in 1482 patients with sub-optimal serum testosterone levels [109]. A similar inverse correlation between serum free testosterone levels and the thickness of carotid intima-media was found in another prospective study involving 196 subjects [110]. The risk of getting atherosclerosis was more severe in elderly patients with higher levels of [111]. Contrarily, Vikan and the team found no such correlation between the low serum testosterone levels and carotid atherosclerosis [112] during a 7 years follow-up. They suggested that the administration of antihypertensive and lipid-lowering drugs could have resulted in a failure in correlating testosterone levels with carotid intima-media thickness. The association between the coronary atheroma and serum testosterone levels is also reported by various studies [113–117]. For instance, the incidence of coronary atherosclerosis [117], coronary calcium score [115], peripheral artery disorders [118] and aortic calcification [113] were found inversely correlated with the sub-optimal levels of total or bioavailable T. The severity of coronary disorders was more adverse in men with sub-optimal levels of total testosterone [116]. Contrarily, such inverse association was not supported by the finding of Basaria and colleagues who claimed no such association between testosterone levels, thickness of carotid intima-media and coronary calcium scores [119]. Likewise, the incidence of developing non-calcified fat deposits was significantly higher in testosterone treated group as compared to the control [120].

2.2.3. Effect on electrocardiograph

The difference of the QT interval between male and female subjects becomes significant only near puberty [121,122] where the interval is comparatively longer in females [123]. This difference between the two sexes is due to the male androgen levels that significantly increase the electrical conductance in the His-Purkinje system that results in the shortening of the QT interval. Zhang and colleagues reported an inverse correlation between serum testosterone levels and QT interval [124]. In addition to the QT interval, the ST segment was also found shorter in men with higher serum testosterone levels [125]. These results were further corroborated by the studies which reported a significant QT prolongation in older men with an age-related decline in serum testosterone levels [126] or in patients who receive androgen deprivation

therapy [127]. Moreover, the TRT is reported to shorten the QT interval in elderly community-dwelling men [128] and chronic heart failure (CHF) patients, without affecting the heart rate [129]. On the account of the higher rate of ventricular tachyarrhythmias and mortalities in patients with longer QT intervals [130–132], the testosterone-induced QT shortening may be taken as a cardioprotective property of testosterone. This notion is further strengthened by the findings of Salem and co-workers that suggested a higher occurrence rate of polymorphic ventricular tachyarrhythmias (torsades de pointes) in men with sub-optimal testosterone levels [133]. Further, the risk of such tachyarrhythmias was attenuated after treating such men with TRT.

2.2.4. Effect on cardiac output and ejection fraction

T therapy has shown significant improvement in older men with compromised cardiac function. For instance, the ejection fraction and cardiac output were significantly improved by transdermal TRT in 76 men with a baseline mean 32.5 % ejection fraction [134]. Moreover, the improvement in the treated and placebo-treated patients was found to be 35 % and 8% respectively [134]. These results were further attested by a subsequent study in 70 congestive heart failure (CHF) patients with an ejection fraction < 40 %. After getting testosterone therapy for almost 6 months, the patients showed a significant improvement in baroreflex sensitivity and aerobic capacity [135]. Since a decline in baroreflex sensitivity is considered as a marker of poor prognosis in CHF [136], an improvement in this may improve the clinical outcomes of CHF therapy in patients receiving TRT. The improvement in cardiac output after oral testosterone therapy can be observed within 2 days of the treatment [137].

It is important to mention here that the TRT is found to exhibit clinically significant fluid retention in older adults with an increased ventricular workload and eventual heart failure [27,138,139]. Nevertheless, testosterone treatment improves the aerobic function of the lungs in elderly patients [140,141] which may attenuate hypoxia-induced tachycardia in such patients.

2.2.5. Antihyperlipidemic effect

Various studies have confirmed an inverse relation of endogenous testosterone levels with serum cholesterol and LDL [142–144]. Some clinical studies have reported no association between testosterone levels and serum triglycerides [27,145,146]. However, some reports have claimed a suppressive effect of serum testosterone on triglycerides levels [142–144]. In addition to LDL (bad cholesterol), the levels of HDL (good cholesterol) are also suppressed by testosterone [142–144,147], although the suppression of LDL was more significant than that of the HDL [145]. This testosterone-induced decline in HDL is because of the enhanced removal of triglycerides as well as phospholipids from these carrier lipoproteins due to the boosted enzymatic activity of hepatic lipase [148]. When testosterone was administration in supraphysiological doses (600 mg per week for three weeks) to elderly eugonadal men, a 66 % increase in hepatic lipase activity was observed with a resultant decline in HDL-cholesterol, HDL-class 2 and 3 [148]. These results were in close agreement with a previous study that claimed an inverse relationship between testosterone treatment and hepatic lipase-induced HDL-cholesterol decline in hypogonadal men [149]. Based on the fact that the endogenous testosterone levels, as well as the TRT, tend to suppress both the good and bad cholesterol, the overall impact of TRT on the cardiovascular risk remains fuzzy. It must also be taken into account that this testosterone-induced decline in serum HDL levels does not affect HDL-mediated efflux of cholesterol in hypogonadal [150] as well as in healthy men [151] up to a significant level.

Interestingly, many clinical studies have reported no impact of testosterone levels/TRT on serum lipoproteins [119,152], yet another study has claimed a decline in serum lipoproteins up to 21 % [153]. Such conflicting reports insist on further investigations to enhance certainty while establishing the correlation between testosterone levels and lipid profiles.

2.2.6. Insulin sensitivity

Reports from various cross-sectional, mechanistic as well as randomized clinical trials have published conflicting results on the correlation between endogenous testosterone levels, metabolic syndrome and insulin resistance. Some of these reports have suggested a preventive effect of testosterone against metabolic syndrome by enhancing insulin sensitivity [153–156], many others negate such a correlation between testosterone and body insulin response [157–159]. Yet a considerable clinical literature claims an inverse relation of the serum testosterone levels with insulin sensitivity and occurrence of metabolic syndrome.

For instance, a relatively higher risk of diabetes and metabolic syndrome in prostate cancer patients who received testosterone deprivation therapy supported a strong correlation between testosterone levels and insulin sensitivity [108,160–166]. Although one such study has claimed a direct correlation between testosterone levels and insulin sensitivity that is independent of body fat [167,168], testosterone was found inversely correlated with the leptin levels [169]. The relevance of endogenous testosterone with obesity and diabetes was further confirmed by Vikan and colleagues who reported a higher occurrence of diabetes and belly white adipose in men with lower serum testosterone [170]. Another study by Antonio and co-workers found a higher incidence of destructive metabolism in patients with lower serum testosterone levels (OR 1.64 CI_{95} 1.41–1.90), suggesting testosterone as an important regulator of metabolism [171]. Moreover, the discontinuation of TRT in patients with sub-optimal testosterone levels has raised the risk of diabetes type 2 by enhancing insulin resistance [172]. Together, these reports produce convincing evidence to enlist endogenous testosterone as one of the many endogenous factors that regulate body fat and glucose metabolism.

2.2.7. Immunomodulatory effect

A chronically higher serum level of inflammatory mediators may pave the way to serious complications involving the cardiovascular system [66,173]. Persistently elevated serum levels of reactive c peptide and cytokines, particularly IL-6 and tumour necrosis factor are proven detrimental for cardiovascular health, even in asymptomatic patients who are more prone to attain serious cardiovascular ailments in future [174–176]. However, studies have reported conflicting results on the correlation between pro-inflammatory mediator levels and endogenous testosterone [169,177–182]. Some of these studies have claimed an inverse relationship between the endogenous testosterone and c-reactive peptide [169,177–180]. Such anti-inflammatory properties of endogenous testosterone are further confirmed by another randomized control trial which found a testosterone-induced expression of the anti-inflammatory cytokine IL-10 [183]. Testosterone also suppressed the expression of the pro-inflammatory cytokines TNFα, IL-1β [183]. Likewise, higher levels of testosterone were found inversely associated with TNF-α and IL-6 [169]. Contrarily, some studies report no association between the endogenous testosterone levels and inflammatory markers [146,181,182].

3. Risk factors associated with endogenous testosterone levels

The correlation between the lower serum testosterone levels and the cardiovascular morbidities/mortalities in young as well as the elderly are well established by the population studies data (Table 1). The following section narrates the findings of epidemiological data on the cardiovascular-related morbidities and mortalities in patients with varying serum testosterone levels to ascertain whether these two truly correlate together. The association between cardiovascular risk and endogenous testosterone levels is assessed by numerous prospective cohort studies. However, these studies have some limitations that must be enlisted before analyzing their finding in detail. Most of these studies have relied only on a single testosterone measurement, ignoring the physiological diurnal changes in the serum testosterone levels during

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

nature of Correlation	Year	Conclusion	Reference
Direct	2014	1251 subjects (age: ≥55) were studied for 10 years. 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)].	[187]
	2014	3690 subjects (age: 70−89) were studied for 9 years. 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.	[188]
	2014	3690 subjects (age: 70−89) were studied for 6.6 years. 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.	[19]
	2013	491 subjects (age: ≤65) were studied for 4 years. 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.	[18]
	2013	581 subjects (age: 31−88) were studied for 5.8 years. The all-cause mortality rate was higher in men with low testosterone levels HR = 2.02 (CI_{95} 1.2−3.4)	[33]
	2012	3637 subjects (age: 70−88) were studied for 5.1 years. 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)].	[189]
	2011	2416 subjects (age: 69−81) were studied for 5 years. 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)].	[17]
	2010	930 subjects (age: ≤60) were studied. 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)].	[190]
	2010	1114 subjects (age: ≥20) were studied for 18 years. 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.	[191]
	2010	1954 subjects (age: 20−79) were studied for 7.2 years. Cardiovascular and the all-cause mortality rate was 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.	[22]
	2009	3443 subjects (ag: ≥70) were studied for 3.5 years. Lower testosterone levels were found associated with the transient ischemic attack and cerebral stroke.	[192]
	2009	1568 subjects (age: average 59.6) were studied for 11.2 years. The high all-cause mortality rate was associated with lower levels of free testosterone levels [HR = 1.24 (CI_{95} 1.01−1.53)].	[193]
	2009	3014 subjects (age: 69−80) were studies for 4.5 years. Higher all-cause mortalities were recorded in patients with lower total testosterone levels [HR = 1.65(CI_{95} 1.29−2.12)].	[194]
	2008	794 subjects (age: 50–91) were studied for 11.8 years. Cardiovascular and the all-cause mortality rate was 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.	[21]
	2007	11,606 subjects (age: 40−79) were studied for 7 years. 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.	[20]
	2006	858 subjects (age: ≤40) were studied for 4.3 years. The higher mortality rate was associated with lower total testosterone levels [HR 1.88 (CI_{95} 1.34−2.63)].	[195]
	2012	182,747 subjects (age: ≥66) were studied for 5.1 years. 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)].	[196]
	2011	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)].	[197]
Indirect (Androgen deprivation therapy in prostate cancer patients)	2011	22,310 subjects (age: ≥40) were studied for 3.9 years. Androgen deprivation therapy was associated with the risk of transient ischaemic attack [RR = 1.18 (CI_{95} 1.00−1.39)].	[198]
	2010	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)].	[162]
	2006	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; $p = 0.004$].	[164]

day and night.

Further, the studies seemed to rely on sub-optimal testosterone values without considering the symptoms of testosterone deficiency in subjects. Besides, the precision and accuracy of the immunoassays used to estimate serum testosterone levels in most such studies are questionable. This factor especially gets more weightage when measuring lower testosterone concentrations [184,185]. The liquid chromatography-tandem mass spectrometry [186], as well as the gold- standard method for testosterone measurement (186), are many appropriate methods for measuring such lower concentrations as compared to the immunoassay techniques.

Over the years, the studies on the possible cardiovascular risks associated with the endogenous testosterone levels have reported contradictory findings. On one side, some reports claim an association between the total testosterone and cardiovascular ailments, yet several studies have reported no association between the two. Moreover, the reported pattern of association between testosterone levels and cardiovascular events is also inconsistent. Many of such studies have

reported the cardioprotective effect of testosterone levels at higher concentrations, approving its endogenous levels as a measure of overall and cardiovascular well-being (in particular) and overall health (in general). Besides, fewer studies have reported a U or J shaped association between the total testosterone levels and cardiovascular events. Such studies have defined a safe concentration range at which the likelihood of cardiovascular morbidities/mortalities were found minimum as compared to their occurrence beyond that range. The findings of these prospective cohort studies are described below.

Numerous reports are claiming a protective effect of endogenous testosterone levels (19–21, 32, 52, 58–63) to prevent deaths related to cardiovascular or some other morbidities [20–22,33,188–191,193–195]. For instance, a study on 2416 elderly Swedish subjects by Ohlsson (16) has reported the cardioprotective effect of testosterone at higher serum levels (≥550 ng/dl) with a 30 % reduced risk of cardiovascular events as compared to the subjects with relatively lower levels of testosterone (16). A decline in the serum testosterone levels below 337 ng/dL was found to double the probability of experiencing a transient ischemic attack or a brain stroke in older men of 70 years age and above [192]. Likewise, Tivesten A and his colleagues conducted a similar study in 3014 elderly Swedish men (62). They found that the risk of all-cause mortality was as higher as 65 % in the first quartile with serum testosterone levels equals or less than 336 ng/dL. However, the death toll was at its significant low at the higher concentrations of the testosterone with a hazard ratio of 1.65 (CI_{95} 1.29–2.12) (62). Although the study employed less accurate immunoassay techniques to access the testosterone levels, their finding was attested by another similar study which used a more accurate spectrometric technique for measuring the testosterone levels [19]. In a nested case-control study involving 2314 subjects, the higher levels of serum testosterone were found associated with a lower death toll [both cardiovascular-disease related (OR 0.53, CI95 0.32 − 0.86) or due to other ailments (OR 0.59, CI95 0.42 − 0.85)] (19). The study divided the data of subjects from 40 to 79 years of age into four quartiles based on their serum testosterone levels, where the mortalities were at their lowest number in the highest quartile. Their findings attested the notion that the endogenous testosterone levels can be taken as a measure of good health. More recently, the findings of a study by Morgunov and colleagues have nominated lower testosterone levels as a contributing factor to ischaemic stroke in type 2 diabetic patients that further corroborate the health benefits of endogenous testosterone levels [199].

The endogenous serum testosterone in men over 70 years of age was found relatively safer at higher serum concentrations, the association was weak [200]. Their work covered 19 prospective cohort and nested case-control studies to evaluate the correlation between the endogenous testosterone levels and the incidence of cardiovascular ailments, including ischemic heart diseases, myocardial infarction, atherosclerosis, and cerebral stroke. The relative risk ratio for cardiovascular diseases in subjects with higher serum testosterone levels was found to be 0.89 (CI_{95} 0.83–0.96) [200]. A similar meta-analysis on the cardiovascular-related mortalities affirmed the relative safety of testosterone at higher serum concentrations [201]. The lower testosterone levels were found associated with cardiovascular-related and all-cause related mortalities with the relative risk of 1.25 (CI_{95} 0.97–1.60) and 1.35 (CI_{95} 1.13–1.62) respectively. Cardiovascular-related and all-cause death In this meta-analysis, lower serum testosterone levels were associated with a higher risk of cardiovascular death (RR 1.25, 95 % CI 0.97–1.60) and all-cause death (RR 1.35, 95 % CI 1.13–1.62) [201]. The findings of these studies are in close agreement with the Framingham Heart Study [187], FINRISK97 study [202] and Cardiovascular Health Study [203] that claimed a significant association of lower endogenous testosterone levels with the incidence of atrial fibrillation.

Numerous reports have claimed a protective effect of endogenous testosterone levels to prevent deaths related to cardiovascular or some other morbidities [20–22,33,188–191,193–195]. However, a few studies did not find such an association between mortality rate and endogenous testosterone levels [204–208]. For instance, higher levels of serum testosterone were found associated with a lower death toll [both cardiovascular-disease related (OR 0.53, CI_{95} 0.32 − 0.86) or other ailments related mortalities (OR 0.59, CI_{95} 0.42 − 0.85)] in a nested case-control study in 2314 subjects [20]. The study divided the data of subjects from 40 to 79 years of age into four quartiles based on their serum testosterone levels, where the mortalities were at their lowest number in the highest quartile. Their findings attest to the notion that the endogenous testosterone levels can be taken as a measure of good health with its concentrations in inverse relation with morbidities or mortalities. Likewise, Tivesten and his colleagues conducted a similar study in 3014 elderly Swedish men [194]. They found that the risk of all-cause mortality was as higher as 65 % in the first quartile with serum testosterone levels equals or less than 336 ng/dL. However, the death toll was at its significant low at the higher concentrations of the testosterone with a hazard ratio of 1.65 (CI_{95} 1.29–2.12) [194].

Contrary to the studies mentioned above that report lower cardiovascular risk associated with the higher testosterone levels, there are a few studies that have claimed a different pattern of association between the two. For instance, the testosterone levels were found safer with the least associated cardiovascular risk at a specific concentration that ranged from 283 to 454 ng/dL in 3690 older men [188]. The association between the two was hence found to follow a U shaped pattern where a shift in testosterone levels below 283 and above 454 ng/dL had raised the cardiovascular risk [188]. A French prospective cohort study has claimed that the testosterone levels exhibit cardioprotective effects within a specific range [18]. However, a change in its concentration either above or below this range was proved to be detrimental for cardiovascular health. They reported a J shaped association between the testosterone levels (both total testosterone and bioavailable testosterone levels) and the cardiovascular risk (ischaemic arterial disorders and coronary heart diseases) in 495 men of 65 years of age and above. The risk of ischaemic arterial disease was minimum in the second quintile (Hazard Ratio 2.23, CI_{95} 1.02–4.88) as compared to the first, third and higher quintiles ($p < 0.01$; Hazard ratio 3.61, CI_{95} 1.55–8.45)

For even a further conflict, there are reports (Table 2) to claim no association between the endogenous testosterone levels and cardiovascular morbidities [193,206,209–212] and mortalities [204–208]. The variations in the total testosterone concentrations in the serum of 1032 men showed no correlation with the occurrence of ischaemic stroke in the subjects [210]. Likewise, the probability of getting ischaemic stroke was found to have no association with the total serum testosterone levels in 1558 subjects during 14.1 years [209].

Further, no causal association was detected between the serum testosterone levels and the mortality due to the cardiometabolic risk factor in 1882 men of 20 − 79 years of age [213]. Rather, the reverse causation or residual confounding was suggested to be the reason behind the testosterone-related cardiotoxicities. There are other studies as well that claim no relation between the testosterone levels and cardiovascular or all-cause mortalities. For instance, a longitudinal cohort study in 1032 elderly subjects (66–97 years of age) reported no association between the cardiovascular-related morbidities/mortalities or all-cause mortalities and total testosterone levels over 9 years to follow up period [205]. However, a non-linear correlation between the incidence of cardiovascular diseases and dihydrotestosterone levels was claimed ($p = 0.04$) [205]. In another study, the mortalities among 1804 middle-aged subjects over 15 years of follow up period seemed associated with lower baseline testosterone levels. However, the association was found statistically non-significant after adjusting the relevant risk factors [204]. This suggestion is further strengthened by the fact that even the largely accepted age-related decline in the serum testosterone levels could be a cause of these residual confounding. Adjustments in such confounding factors such as the comorbidities, obesity, alcohol consumption, and smoking have proved to make the association between the age and testosterone levels non-significant [4].

Succinctly, the epidemiological studies have strengthened the

Table 2
Studies claim no association between cardiovascular diseases and endogenous testosterone levels in elderly men.

Sr. NO	Year	Conclusion	Reference
1	2016	No association was found between the incidence of atherosclerosis-related brain stroke and the endogenous testosterone levels in a study of 1558 men from 51 to 76 years of age.	[209]
2	2016	The cardiovascular or all-cause mortality risk was not associated with total testosterone levels.	[204]
3	2014	A nonlinear association between the levels of dihydrotestosterone and stroke risk was found in 1032 men of 66 years of age and above. However, total and free testosterone levels were not associated with stroke incidence.	[210]
4	2014	The cardiovascular or all-cause mortality risk was not associated with total testosterone levels.	[205]
5	2013	In a study on 254 men with an average age of 75.5 years, the cardiovascular morbidities, including infarction, coronary insufficiencies, and congestive cardiac failure were not found associated with total testosterone levels.	[206]
6	2013	All-cause mortality risk was not found associated with higher baseline total testosterone levels at ten years follow up period.	[206]
7	2009	A study on 1318 men with an average age of 59.6 years reported non-significant relation between the incidence of myocardial infarction and the total and free testosterone levels.	[193]
8	2009	The all-cause mortality risk was not associated with the total testosterone levels.	[207]
9	2007	No association between the incidence of stroke and total testosterone levels was reported in 2197 men with age ranging from 71 to 94 years.	[211]
10	2007	The all-cause mortality risk was not associated with the total testosterone levels.	[208]
11	2006	The incidence of cardiovascular problems including angina, coronary abnormalities, stroke was not found associated with the total testosterone levels in 2084 men with ages between 30–60 years.	[212]

notion that the endogenous testosterone levels could be taken as a measure of good health. Despite some contradictions among their findings, most of the cohort studies have attributed the lower testosterone levels as the risk factor for cardiovascular ailments including ischemic heart diseases, arterial morbidities such as atherosclerosis, stroke and all-cause deaths. These findings are supported by the fact that the androgen deprivation therapy in prostate cancer patients has resulted into serious cardiovascular adversities in these patients, including diabetes, myocardial infarction, peripheral arterial disorders, coronary heart diseases, venous thromboembolism, sudden cardiac death, transient ischemic attack [162,164,196–198]. However, the serum testosterone levels in such patients decline below the castrate range (50 ng/dL) that is far below the low cut off the range in most of the cohort studies that we have discussed above. Yet, the alarming cardiovascular risk factors associated with the androgen deprivation therapy support the claim that testosterone exhibits cardioprotective impact in its normal physiological range.

4. Epidemiological studies on testosterone therapy

In men with suboptimal testosterone levels, TRT has been associated with a higher risk of cardiovascular adversities as reported by various retrospective studies over the past many years. Yet many of such studies have reported no association between the cardiovascular adversities and TRT. One must consider the limitations (if any) associated with these findings that may account for precipitating into misleading inferences. Moreover, the final judgment on the subject should not be based on the findings of either of these reports. Instead, the inferences derived from such retrospective studies must be compared with similar other studies to identify any limitation affecting their judgment. For instance, the finding of a study by Vigen and co-workers suggested that a higher risk of myocardial infarction, stroke, and all-cause mortality was associated with TRT [23]. Firstly, all the enrolled 8709 subjects in the study were those who underwent coronary angiography. Hence, the study was conducted into a special population of patients and its findings could not be equally applied to the general population. Secondly, the study claimed that 1223 out of the enrolled 8709 subjects had received TRT on account of their low testosterone levels. The therapy resulted in a significantly higher risk of myocardial infarction, stroke and mortalities in these patients that was 29 % higher than the other patients with low testosterone levels who did not receive such replacement therapy. However, they ignored the fact that the mean testosterone levels of the patients who received TRT and who did not receive this therapy were 175 ng/dL and 205 ng/dL respectively. The patients who received testosterone therapy had significantly lower testosterone levels than the other group. An adjustment to counter this difference in the baseline testosterone levels might have improved the accuracy and reliability of the findings.

In order to identify an association between the cardiovascular ailments and TRT, Finkle and colleagues conducted a study on 55,593 subjects who were prescribed with TRT [24]. Their finding suggested that the risk of getting non-fatal myocardial infarction was increased up to 36 % within 90 days of receiving intramuscular TRT compared with its likeliness to occur before testosterone administration. In those patients who did not refill their prescription, this risk factor gradually ameliorated in 91–180 days of the initial administration. Based on these findings, the study associated the risk of non-fatal myocardial infarction with TRT [24]. However, these findings should be reconsidered under the light of another similar study that associated the risk of myocardial infarction with the very first testosterone administration only [25]. From a larger sample size (934,283) of subjects (45–80 years age), Etminan and coworkers reported no association between the TRT and the risk of myocardial infarction in patients who were already on this therapy. However, the risk factor was significantly associated with the first administration of testosterone [RR = 1.41 (CI_{95} 1.06–1.87). Under the light of these findings, the risk of myocardial infarction in patients who did not refill their testosterone prescription in Finkles' study could have a different outcome in case of continuing their testosterone therapy. Secondly, Finkleclaimed that the associated with was in elderly patients. In patients aged 65 or above, the likelihood of non-fatal myocardial infarction was enhanced by up to 119 %.

Further, the risk was 243 % in elderly patients with age ≥75. Finkle also claimed that the risk of non-fatal myocardial infarction was also significantly higher in those younger subjects who had a previous history of cardiovascular ailments. Based on these findings, it was suggested that this alarmingly higher increase in cardiovascular adversities is associated with the TRT, particularly in elderly patients and in younger subjects with a previous history of cardiovascular malady. However, the findings of another parallel study by Maggi and colleagues [214] should be taken into account before considering Finkles' claim. In their study involving 999 subjects, Maggi and the team found no association between the TRT and cardiovascular risk. Instead, the risk of myocardial infarction in the enrolled subjects was found associated with their age and previous history of myocardial disorders [214].

Contrary to the studies that either claimed association or no association between the TRT and cardiovascular risk, there are many reports to claim a positive impact of such therapy on the cardiovascular and overall well-being of the subjects. For instance, the findings of an observational study by Haider and colleagues have claimed a negative correlation between exogenous testosterone therapy and body mass index [215]. Such potential health benefit of testosterone therapy was found more prominent in subjects who underwent physical exercise along with testosterone therapy [216]. The mortality rate in patients

who received testosterone therapy (10,311 subjects) was found significantly less [HR = 0.88 (CI_{95} 0.84 − 0.93)] as compared to the controls (28,029) [38]. Likewise, the risk of all-cause mortality was significantly reduced in patients whose testosterone levels were normalized due to exogenous testosterone therapy when compared with control [217]. The risk was even more reduced in patients who were exposed to the therapy for a longer duration (35 months) of time [HR = 0.67 (CI_{95} 0.62 − 0.73)]. Besides the mortality rate, the incidence of cardiovascular morbidities including myocardial infarction, venous thromboembolism, and stroke was also found at significantly low in patients who had longer exposure to testosterone therapy [HR = 0.84 (CI_{95} 0.72 − 0.98)].

Moreover, the risk of getting these cardiovascular morbidities was significantly higher [HR = 1.26 (CI_{95} 1.09–1.46)] in patients who received testosterone therapy for a shorter period (60 days). These findings supported the claim of previously discussed studies [24,25,214] that the cardiovascular morbidities and mortalities in patients receiving TRT could be reduced the control groups if the therapy is prolonged for a certain. However, taking such therapy for a shorter period may precipitate into a higher risk of cardiovascular and all-cause mortalities and morbidities, particularly in elderly patients and in younger individuals with a previous history of cardiovascular ailments.

Following the epidemiological data on endogenous testosterone levels, as discussed above, the inference derived from the studies mentioned above requires one further addition. There is a likelihood that the patients on TRT do not respond to it and their serum testosterone levels remain sub-optimal even after receiving the testosterone therapy. Many epidemiological reports have attributed endogenous testosterone within its optimal levels as a measure of good health. The study of Sharma and colleagues has suggested that the testosterone therapy-associated cardiovascular risk is raised in those patients whose testosterone levels remain sub-optimal even after receiving the therapy [35]. On the other hand, the therapy reduced the risk of myocardial infarction, stroke and all-cause deaths in another group of individuals whose testosterone levels were returned back to the optimal range by TRT. In another similar study on 4736 subjects, the occurrence of cardiovascular disorders was at its significant low [HR = 0.74 (CI_{95} 0.56 − 0.98); $p = 0.04$] in patients whose testosterone levels were raised to normal range after testosterone therapy [37]. Moreover, the mortality rate was recorded at its maximum [HR = 0.65 (CI_{95} 0.47 − 0.90)] in patients whom testosterone levels remained sub-optimal even after receiving exogenous T. These reports were further supported by the findings of Vigen and colleagues where the baseline serum testosterone levels were significantly lower in the high-risk group as compared to the control [23].

In addition to the non-responding serum testosterone levels as suggested by Anderson and Sharma's findings, smoking was also found involved in enhancing the risk of all-cause mortalities and myocardial infarction in patients who received testosterone therapy [39]. Moreover, one can relate the cardiovascular outcomes of testosterone therapy with the serum testosterone levels achieved in the patients as evident from the studies mentioned above [35,37]. Hence, the dosage form used to administer testosterone should also be considered. It is likely possible that the serum testosterone levels are raised more efficiently and abruptly in patients receiving intramuscular testosterone injections compared with those who are given transdermal sustained release gel formulations. A study on 544,115 men aged 18 years and above has found a similar relation between the dosage form and the safety profile of testosterone therapy. The risk of hospitalization [HR = 1.16 (CI_{95} 1.13–1.19)], mortalities [HR = 1.34 (CI_{95} 1.15–1.56)] and cardiovascular morbidities [HR = 1.26 (CI_{95} 1.18–1.35)] was found significantly higher in individuals who received intramuscular testosterone as compared to those who received transdermal gel preparation [218].

Summarizing the findings of the above mentioned prospective studies, the cardiovascular safety of TRT should be observed under the light of many important factors such as the age, previous medical history, baseline serum testosterone levels, outcome of testosterone therapy in terms of serum testosterone levels, dosage form, and smoking.

5. Clinical trials

The published results from the clinical trial until today are insufficient to answer many important questions in relating the cardiovascular risk with the TRT in hypogonadism. Secondly, the available data from the published trails lack various standards while reporting the safety profile of testosterone therapy [219]. The reported findings of clinical trials are briefly discussed in the following section.

The administration of micronised testosterone (600 mg/day) in 221 patients with alcoholic cirrhosis was the first clinical study on testosterone therapy that published its findings in 1986 [220]. The study was aimed to evaluate the impact of testosterone therapy on the survival rate in such patients. On account of the higher fatality rate in the enrolled subjects, the study had to quit before time with a median follow up period of fewer than three years. Importantly, only one out of the total enrolled patients died because of the cardiovascular ailment. Hence, the study did not associate cardiovascular risk with testosterone therapy, albeit it provided the first-ever evidence of testosterone-related fatality.

The very first trial to report the cardiovascular-related risk of testosterone therapy published its results in 2010 [27]. The study enrolled a small sample size of 209 community-dwelling older men with a limitation of mobility. All enrolled subjects were older than 65 years with a mean age of 74 years. The baseline serum total testosterone levels and free testosterone levels of the enrolled subjects were 100–300 ng/dL and less than 50 pg/mL respectively. The subjects were divided into the treatment and placebo groups and a single daily dose of 100 mg was administered to the treatment group for 6 months through transdermal testosterone gel. Owing to the higher mortality rate in the treatment group, the study had to quit earlier. The death ratio between the treatment and placebo groups was 4.6:1. The cardiovascular adversities in the treatment group included both atherosclerotic and non-atherosclerotic disorders. Besides, respiratory and dermatological adverse effects were also observed in the treatment group. Above all, the serious cardiovascular events that included two myocardial infarctions, one stroke and one cardiovascular-related death were observed only in the treatment group.

Although the findings of the trial were alarming, its results could not be related to the general population because of a few limitations, with the smaller sample size being the first. Secondly, more than 80 % of the subjects had hypertension. Nearly half of the subjects were obese with a previous history of cardiovascular ailments, and almost 25 % had diabetes mellitus. Such a higher prevalence of comorbidities in the enrolled subjects could not be ignored [221,222]. Moreover, the age of all the studied subjects was more than 65 years, ignoring the fact that elderly patients are more prone to such testosterone therapy-associated cardiovascular apathy [214]. Later, Basaria and co-workers published another analysis on the TOM trial to claim that the testosterone therapy associated higher cardiovascular risk was attributed to the higher free testosterone levels in serum [138]. Many of the important co-factors were not taken into account while relating the abrupt appearance of cardiovascular events within weeks of the initiation of testosterone therapy. Later studies proved the significance of age, history of previous cardiovascular ailments and smoking that had to be considered in the TOM trial [24,25].

Following their findings published in the TOM trial in 2010 and 2013 [27,138], Basaria and co-workers conducted their study to evaluate the role of testosterone therapy in developing subclinical carotid atherosclerosis in older men aged 60 or above. They published their findings as a TEAAM trial in 2015 [119]. All of the 308 enrolled subjects had their total serum testosterone levels within the range of

Table 3
Meta-analyses on the association between cardiovascular risk and TRT. Abbreviations used in the table are 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; CI95: 95 % confidence interval. Symbols used are; A: associated; CA: conditionally associated; NA: not associated.

Year/ Reference	CV risk : Testosterone Therapy Association	n_s	Age	Cardiovascular Events/ Mortalities		Remarks
				Treatment	Placebo	
2018 [227]	**NA**	108 (15 + 93)[a]	< 65	E_{cv}/n_t 18/2037	E_{cv}/n_t 14/1331	No association between MACV and testosterone therapy (OR 0.97 CI_{95} 0.64 − 1.46)
			≥65	E_{cv}/n_t 56/3476	E_{cv}/n_t 48/2713	
2017 [228]	**NA**	30	42 − 79	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)
2016 [231]	**CA**	45	56 − 72	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).
2013 [28]	**A**	27	24 − 87	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).
2010 [229]	**NA**	51	18 − 88	E_{cv}/n_t 66/1750	E_{cv}/n_t 43/1226	No significant association between cardiovascular risk and testosterone therapy.
2007 [230]	**NA**	6	≥40	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)
2005 [226]	**NA**	19	≥45	E_{cv}/n_t 18/651	E_{cv}/n_t 16/433	(OR = 1.14, CI95 0.59 − 2.20)

[a] Out of 108 studies included in the meta-analysis, 93 were controlled trials and 15 were pharmaceutical funded epidemiological studies.

100–400 ng/dL whereas their free serum testosterone was less than 50 pg/mL. The subjects were divided into treatment and placebo groups. The treatment group received 75 mg/day testosterone through transdermal gel formulation for 3 years. No association between testosterone therapy and the progression of atherosclerosis was observed.

Moreover, the coronary artery calcium scores provided no evidence to support the involvement of testosterone therapy in developing calcified plaques in coronary arteries. No significant difference in carotid intima-media thickness was observed between the treatment and placebo groups. Importantly, treatment group versus placebo ratio of coronary revascularisation, myocardial infarction, stroke and cardiovascular-related mortality was found to be 5:2, 3:2, 3:0 and 1:0 respectively. Although testosterone therapy was not associated with the initiation and propagation of atherosclerosis, the number of cardiovascular events was found more in the treatment group as compared to the placebo [119].

T trials refer to a group of multicenter placebo-controlled double-blind trials that published their findings between 2016 and 2017 [9,120,223–225]. The trial involved multiple groups of researchers worldwide who coordinated together to evaluate the impact of testosterone therapy on overall physical fitness, cognition, sexual functions, vitality, bone mass development, and anaemia. The initial trial included 790 elderly subjects aged 65 years and above with their serum testosterone levels below 275 ng/dL [9]. The treatment subjects were given 50 mg testosterone daily through the transdermal gel for 1 year. Out of these 790 subjects, a sub-group of 138 was subjected to computed-tomographic angiography to evaluate the impact of testosterone therapy on calcified and non-calcified plaque volumes and calcium scores in coronary arteries [120]. No association was found between testosterone therapy and calcified plaque volumes. However, the non-calcified plaque volumes were significantly different between the treatment and placebo groups. No cardiovascular hazard was found associated with testosterone therapy and the incidence of myocardial infarction, stroke and cardiovascular-related mortalities were found equal in number between the two groups [9,120].

Another clinical trial with the name the “TRAVERSE” trial is initiated in 2018 with 6000 enrolled men (age: 45–80; serum testosterone levels < 300 ng/dL) with high cardiovascular risk [41]. The patients will receive either testosterone gel or placebo for five years, and the impact of testosterone treatment on cardiovascular parameters will be evaluated. Being the very first randomized control trial, the trial is expected to produce a breakthrough in answering the quarries and ambiguities which have not been solved so far. The trial may provide substantial data to suggest the association between cardiovascular events and testosterone therapy, including the frequencies of arterial disorders, stroke, myocardial infarction and the ratio between fatal and non-fatal myocardial infarction between the treatment and placebo groups. However, the trial may take several years ahead to produce credible results to ascertain testosterone-associated cardiovascular risk.

6. Meta-analyses of randomized trials

Many research groups have gathered and analysed the available data from controlled and randomized clinical trials on TRT to claim a “significant” association between testosterone therapy and its cardiovascular adversities. However, one must consider the fact that most of these clinical trials have produced conflicting results as we have already discussed above. The cardiovascular events that were recorded in the treatment group in a given clinical trial were equally reported in the placebo group in another trial. Secondly, most of these trials enrolled elderly patients, ignoring the fact that the patients in older ages are more prone to cardiac. Above all, such clinical trials enrolled patients with the previous history of cardiovascular ailments. Hence their findings cannot be applied to the general population with younger patients or healthy individuals without pre-existing cardiovascular disorder. inference these analyses. The findings of these meta-analyses are summarised in Table 3.

As evident from Table 3, most of these meta-analyses reported no association [226–230], one study reported conditional [231] whereas another claimed significant association [28] between testosterone therapy and cardiovascular morbidities. Calof and co-workers analysed 19 clinical trials on middle-aged patients age 45 years and above [226]. The patients in the treatment group (n = 651) received testosterone therapy for 90 days. The number of cardiovascular events observed in 651 treated and 433 placebo subjects was 18 and 16 respectively (OR 1.14, CI95 0.59 − 2.20). Another study analysed six clinical trials where the number of observed cardiovascular events was 14 and 7 in a total of 161 treated and 147 placebo subjects respectively (OR 1.84, CI95 0.78 − 4.23) [230]. The fatal to the non-fatal ratio for myocardial infarction events was 2.24 (CI95 0.50 − 10.02). None of these studies

reported association between cardiovascular risk and testosterone therapy. Likewise, Fernandez and colleagues analysed 51 randomized and non-randomized clinical trials with a total number of 2679 subjects enrolled [229]. Their findings also claimed no cardiovascular risk for patients receiving TRT. The conclusion of the above mentioned meta-analyses was further affirmed by another two recent meta-analyses that also reported no cardiovascular related events in testosterone receiving patients [227,228].

Contrarily, the study of Xu-Freeman and colleagues reported a 54 % increase in cardiovascular events in testosterone-receiving patients [28]. The association between treatment and the cardiovascular hazard was found statistically significant (OR 1.54, CI_{95} 1.09–2.08). Their findings also revealed a conflicting pattern of results between the pharmaceutical-funded and non-funded clinical trials. The trials that were funded by pharmaceutical companies reported significantly lower cardiovascular risk (OR 0.89, CI_{95} 0.50–1.60) in treated patients as compared to those trials which did not receive pharmaceutical funding (OR 2.06, CI_{95} 1.34–3.17). Similarly, another study reported testosterone therapy-associated cardiovascular events in elderly patients (age: $\geq$65; RR 2.90, CI_{95} 1.35 − 6.21), although their findings suggested no such association in younger patients (RR 1.10 CI_{95} 0.86 − 1.41) [231]. In addition to age, the risk factor was related to the duration of treatment and the rout of testosterone administration. Cardiovascular risk was found greater in the first year (RR 1.79 CI_{95} 1.13–2.83) as compared to the later years of the treatment. Likewise, the risk was greater in patients who received transdermal therapy (RR 2.80 CI_{95} 1.38–5.68) as compared to those who received intramuscular treatment (RR 0.96 CI_{95} 0.462–1.98). These results conflicted with a previous study that found intramuscular testosterone therapy riskier as compared with transdermal gel [218].

7. Conclusions

Besides its primary role in male secondary sex character, fertility, and sexual behaviour, testosterone plays a significant regulatory role in metabolism, growth and cardiovascular functions. Testosterone deficiency may result in Hypogonadism and other serious health disorders in the elderly as well as young men. Over the past few decades, the number of testosterone prescriptions has been alarmingly increased around the globe to treat hypogonadism. Thus far, the available reports including epidemiological studies, clinical trials and meta-analyses have generated conflicting opinions in establishing an association between cardiovascular adversities and TRT. According to some retrospective assessments, TRT exerts advantageous effects on mortality. Other studies including some randomized trials, have associated the likeliness of severe cardiovascular risks with TRT. Succinctly, the imperative shortcomings in the study design, selection criteria and result analyses of most of such clinical trials and epidemiological studies may account for such conflicting findings. Besides, none of the published documents on TRT trials have been sufficiently powered to evaluate cardiovascular issues. Consequently, the TRAVERSE trial was initiated in 2018 to evaluate the cardiovascular risks/safety of TRT. The study is designed for five years to get a better understanding of this context. Meanwhile, to make smart decisions, clinicians should openly discuss with their patients about the prevailing data on the cardiovascular risks of TRT.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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