

Chapter 3

The Roles of Testosterone in Cardiac Ischemia/Reperfusion Injury



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Abstract Cardiac ischemia/reperfusion (I/R) injury is a serious cardiac complication following acute myocardial infarction, which include myocardial infarct size expansion, left ventricular (LV) dysfunction, and fatal cardiac arrhythmias. Sex hormone deprivation due to aging or other pathological conditions is an independent risk factor for cardiovascular disease. Several scientific research studies have been conducted to investigate the role of sex hormones in myocardial injury during cardiac I/R. Testosterone is the primary sex hormone in men; it regulates male sexual characteristics, and controls muscle and bone mass. In addition, testosterone plays an important role in regulating LV function through multiple mechanisms, including those controlling cellular calcium homeostasis, regulating cardiac mitochondrial function and enhancing antioxidants. Findings from studies regarding the roles of testosterone on the heart during cardiac I/R are controversial; some have reported that decreased testosterone level could impair LV function, whilst others reported the benefits of testosterone deprivation during cardiac I/R. In this chapter, we include evidence regarding the effects of testosterone deprivation and exogenous testosterone administration on myocardial injury in terms of myocardial infarct size, LV function, arrhythmias and molecular alterations. Reports from in vitro, ex vivo, in vivo studies, and clinical reports are summarized and discussed. The contents of this chapter will explain the roles of testosterone during cardiac I/R in preventing cardiac complications. Insights from these reports may help to devise strategies to improve treatment in patients with acute myocardial infarction.

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Introduction

Acute myocardial infarction (AMI) remains a global leading cause of death [1]. It is caused by narrowing of the coronary arteries. It has been demonstrated that risk factors associated with AMI are atherosclerosis, hypertension, high cholesterol, diabetes, obesity, smoking and hormonal imbalance [2]. Reperfusion therapy is an effective treatment for AMI as it allows the return of blood to the injured myocardium to resupply essential oxygen and nutrients. However, this intervention has been shown to cause potentially serious adverse effects on the heart, including myocardial infarct size expansion, left ventricular (LV) dysfunction and fatal cardiac arrhythmias, collectively known as cardiac ischemia/reperfusion (I/R) injury [3].

It has been demonstrated that sex hormone dysregulation is associated with the severity of coronary artery disease [4]. Testosterone is a male sex steroid hormone which is secreted mainly by the testicles and, to a lesser extent, by the adrenal glands. Testosterone regulates the development of male reproductive organs and promotes secondary sexual characteristics [4]. In addition, it controls hair growth, muscle growth, collagen synthesis, muscle strength and endurance, memory function, mood, red blood cell production and body mass density [5]. Testosterone deprivation, as seen in male aging, has been associated with the pathophysiology of various organ dysfunctions [4]. In the case of the heart, testosterone deprivation increases the risk of cardiovascular disease by increasing total plasma cholesterol levels and reducing plasma high density lipoprotein (HDL) levels [6]. A report from a clinical study demonstrated that ST–Elevation Myocardial Infarction (STEMI) male patients with hypotestosteronemia had a higher incidence of microvascular obstruction as identified by coronary angiography, compared to STEMI patients with normal plasma testosterone [7]. These findings suggest that testosterone may potentially be involved in the pathogenesis of AMI in male subjects.

In order to understand the mechanisms employed by testosterone in the heart, an animal model of testosterone deprivation has been established. In male rodents, a bilateral orchiectomy (ORX) has been shown to eliminate the endogenous production of testosterone [8, 9]; exogenous testosterone being subsequently given to those rodents as therapy [10, 11]. Exogenous testosterone replacement therapy has been widely used to treat patients with hypogonadism and other low–testosterone related diseases [12]. It has been shown that exogenous testosterone offers various benefits for the human body, for instance it augmented positive mood, increased sexual motivation and performance, increased muscle strength and a lean body mass, reduced body fat mass and improved bone mineral density [5]. However, exogenous testosterone has also been shown to cause adverse effects such as acne, mild fluid retention, increased urination symptoms and worsening of sleep apnea [13]. In accordance with

these findings, the Endocrine Society do not recommend treatment with exogenous testosterone in men with heart failure (NYHA class III or IV) [14].

In this chapter, we have summarized and discussed the cumulative data from *in vitro*, *ex vivo*, and *in vivo* studies, and from clinical reports regarding the effects of testosterone deprivation and exogenous testosterone administration on the heart during cardiac I/R injury. This information will provide mechanistic insight for future clinical investigations.

The Effects of Testosterone Deprivation on the Heart with Cardiac I/R Injury

Previous reports have demonstrated that cardiac I/R injury led to myocardial infarct size expansion, LV dysfunction and arrhythmia [10, 15–17]. Cardiac I/R injury has been induced in both *ex vivo* and *in vivo* settings [18]. For induction in the *ex vivo* setting, the heart was removed and connected to a Langendorff system [18]. The myocardial ischemia was induced by either stopping the perfusate for several minutes to induce global ischemia or ligating the left coronary artery to induce regional ischemia [18]. After successful myocardial ischemia, the perfusate was opened to initiate reperfusion to the injured myocardium [18]. In the *in vivo* setting, a thoracotomy was performed and the left coronary artery or left anterior descending (LAD) coronary artery was ligated to induce myocardial ischemia and reperfusion was induced by a loosening of the knot [8, 10, 15]. The alterations in the electrocardiogram were used to confirm a successful cardiac I/R *in vivo* [8, 10, 15].

The bilateral ORX is a surgical procedure effective in causing a decrease in plasma testosterone level in the body prior to the induction of cardiac I/R [10]. Findings regarding the effects of testosterone deprivation on the heart with I/R injury are inconsistent; some studies have reported that testosterone deprivation led to an aggravation of myocardial damage after cardiac I/R [10, 19–23], whereas several other studies reported contrary findings [24–32]. A comprehensive summary of these reports is shown in Table 3.1.

With regards to the deteriorating effects caused as a result of testosterone deprivation on the heart with I/R, results from *ex vivo* and *in vivo* studies have shown that testosterone deprivation for at least 2 weeks increased myocardial infarct size after cardiac I/R [10, 21–23]. Testosterone deprivation for 3 weeks did not alter LV function [19], however, a worsening of LV dysfunction was initially observed after 7 weeks of ORX as indicated by increased LV end-diastolic pressure (LVEDP), reduced LV developed pressure (LVDP) and \pm dP/dt in *ex vivo* studies [20, 21, 23]. In an *in vivo* study, after 12 weeks of ORX, increased LVEDP and $-dP/dt$ and decreased stroke volume (SV) were observed [10]. Cardiac arrhythmia following cardiac I/R was investigated in two studies. These both found that the arrhythmia

Table 3.1 The effects of testosterone deprivation on the heart with cardiac I/R injury, in comparison with normal testosterone level

Animal model/ Duration of hormone depletion	I/R protocol	Findings				Interpretation	Refs.
		Infarct size	LV function	Arrhythmias	Molecular findings		
ORX rats 3 weeks	Ex vivo Global ischemia 20 min/45 min	N/A	- ↔ Coronary flow, Aortic flow, CO, Cardiac work, LVDP, ± dp/dt, HR, SBP, DBP	N/A	- ↔ LDH, MnSOD, GPX - ↓ Catalase	Testosterone deprivation did not alter LV function, but it reduced antioxidants in rats with cardiac I/R	[19]
ORX rats 7 weeks	Ex vivo Global ischemia 20 min/40 min	N/A	- ↑ LVEDP - ↓ LVDP	N/A	- ↑ Intracellular Ca ²⁺	Testosterone deprivation aggravated LV dysfunction by increasing intracellular calcium levels in rats with cardiac I/R	[20]

(continued)

Table 3.1 (continued)

Animal model/ Duration of hormone depletion	I/R protocol	Findings				Interpretation	Refs.
		Infarct size	LV function	Arrhythmias	Molecular findings		
ORX rats 8 weeks	Ex vivo LAD ligation 30 min/120 min	↑	- ↔ Coronary flow, HR - ↓ LVDP, ± dP/dt	N/A	- ↑ LDH - ↔ HSP70, HSF1	Testosterone deprivation increased myocardial infarct size and aggravated LV dysfunction in rats with cardiac I/R, but it was not involved in HSP pathway	[21]

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Table 3.1 (continued)

Animal model/ Duration of hormone depletion	I/R protocol	Findings				Interpretation	Refs.
		Infarct size	LV function	Arrhythmias	Molecular findings		
ORX rats 9 weeks	Ex vivo Left coronary ligation 30 min/120 min	↑	- ↑ LVEDP - ↓ LVDP, ± dP/dt	-↑ PVB, VT	-↑ LDH	Testosterone deprivation increased infarct size, arrhythmias, and aggravated LV dysfunction in rats with cardiac I/R	[23]
ORX rats 15 days	In vivo Left coronary artery ligation 60 min/4 h	↑	N/A	N/A	N/A	Testosterone deprivation increased infarct size in rats with cardiac I/R	[22]

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Table 3.1 (continued)

Animal model/ Duration of hormone depletion	I/R protocol	Findings				Interpretation	Refs.
		Infarct size	LV function	Arrhythmias	Molecular findings		
ORX rats 12 weeks	In vivo LAD ligation 30 min/120 min	↑	- ↑ LVEDP, - dp/dt - ↔ HR, LVESP, Pmax, min + dp/dt - ↓ SV	- ↑ Arrhythmia score - ↓ Time to 1st VT/VF onset	- ↑ Bax/Bcl2 ratio - ↑ Mito ROS, Mito depolarization, Mito swelling - ↔ Procaspase 3 - ↓ p-Cx43 ^{ser368} /Cx43	Testosterone deprivation increased infarct size, arrhythmias and aggravated LV dysfunction by reducing gap junction proteins, increasing apoptosis, and cardiac mitochondrial dysfunction in rats with cardiac I/R	[10]

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Table 3.1 (continued)

Animal model/ Duration of hormone depletion	I/R protocol	Findings			Interpretation	Refs.
		Infarct size	LV function	Arrhythmias		
ORX rats 4 weeks	Ex vivo Global ischemia 20 min/30 min	N/A	- ↔ LVDP	N/A	- ↔ TUNEL ⁺ cells	[27]
ORX mice 20–32 weeks	Ex vivo Global ischemia 90 min/30 min	↓	- ↑ LVDP, ± dP/dt, RPP - ↔ Coronary flow - ↓ Magnitude of contracture	N/A	N/A	[24]

(continued)

Table 3.1 (continued)

Animal model/ Duration of hormone depletion	I/R protocol	Findings				Interpretation	Refs.
		Infarct size	LV function	Arrhythmias	Molecular findings		
ORX rats 3 weeks	Ex vivo Left coronary artery ligation 30 min/150 min	↓	N/A	N/A	- ↔ p-AMPK/AMPK, p-mTOR/mTOR, RAGE -↓ Beclin1, Atg5,12, LC3II/LC3I, Cleaved caspase9,3	Testosterone deprivation reduced infarct size by reducing autophagy and apoptosis in rats with cardiac I/R	[28]
ORX rats 4 weeks	In vivo LAD ligation 25 min/40 min	N/A	N/A	N/A	-↓ myocardial TNFα, IL1β, ICAM1 -↓ Cardiac troponin I	Testosterone deprivation reduced cardiac inflammation and injury in rats with cardiac I/R	[25]

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Table 3.1 (continued)

Animal model/ Duration of hormone depletion	I/R protocol	Findings			Interpretation	Refs.
		Infarct size	LV function	Arrhythmias		
ORX rats 7 weeks	Ex vivo Global ischemia 25 min/40 min	N/A	-↑ LVDP ± dP/dt	N/A	-↑ p-Akt/Akt -↑ p-Bad, Bax, Bim, Bcl2 MnSOD -↓ FasL	Testosterone deprivation attenuated LV dysfunction by activating survival proteins and antioxidants in rats with cardiac I/R [26, 29, 32]
ORX rats 7 weeks	Ex vivo Global ischemia 25 min/40 min	N/A	-↑ LVDP ± dP/dt -↓ LVEDP	N/A	-↓ p-p38/p38, Caspase 1, Caspase 3	Testosterone deprivation attenuated LV dysfunction by reducing cellular stress, inflammation, and apoptosis in rats with cardiac I/R [31]

(continued)

Table 3.1 (continued)

Animal model/ Duration of hormone depletion	I/R protocol	Findings			Interpretation	Refs.
		Infarct size	LV function	Arrhythmias		
ORX rats 12 weeks	In vivo LAD ligation 5 min/5 min	N/A	-↓ Cardiac rate BP	N/A	-↑ Mito respiration -↔ Mito Ca ²⁺ transport, Mito membrane potential -↓ Muscle fiber separation	[30]
ORX rats with HFD induced obese-insulin resistance 12 weeks	In vivo LAD ligation 30 min/120 min	↔	-↑ LVEDP -dP/dt -↔ HR Pmax, min -↓ LVESP SV	-↔ Arrhythmia score Time to 1st VT/VF onset	-↔ p-Cx43 ^{ser368} /Cx43 -↔ Mito ROS, Mito depolarization, Mito swelling	[10]

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Table 3.1 (continued)

Animal model/ Duration of hormone depletion	I/R protocol	Findings				Interpretation	Refs.
		Infarct size	LV function	Arrhythmias	Molecular findings		
ORX rats with obesogenic diet induced obese—insulin resistance 20 weeks	In vivo LAD ligation 30 min/120 min	↔	N/A	N/A	N/A	Testosterone deprivation did not increase infarct size in obese—insulin resistant rats with cardiac I/R, compared with their sham operation controls	[37]

I/R: ischemia/reperfusion; ORX: orchietomy; LV: left ventricular; LAD: left anterior descending coronary artery; CO: cardiac output; LVDP: left ventricular developed pressure; LVEDP: left ventricular end diastolic pressure; LVES: left ventricular end systolic pressure; RPP: rate pressure product; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; LDH: lactate dehydrogenase; MnSOD: manganese superoxide dismutase; GPX: glutathione peroxidase; Ca²⁺: calcium; HSP: heat shock protein; HSF: heat shock factor; PVB: premature ventricular beat; VT: ventricular tachycardia; VF: ventricular fibrillation; Mito: mitochondria; ROS: reactive oxygen species; Cx: connexin; TNF: tumor necrosis factor; IL: interleukin; N/A: not assessed

score [10], the number of premature ventricular beats (PVB) and ventricular tachycardia (VT) increased after 9 weeks of ORX [23]. The mechanisms underlying the deteriorating effects of testosterone deprivation on cardiac I/R were determined, and the data showed that testosterone-deprived rats had an increase in lactate dehydrogenase (LDH) [21, 23], apoptosis and cardiac mitochondrial dysfunction [10]. In addition, gap junction protein expression was decreased in ORX rats when compared to their sham operation controls [10], leading to a disturbance of electrical activity in the heart. A comprehensive summary of these reports is shown in Table 3.1.

Mitochondria are vital organelles which control energy metabolism, cell death and cell survival in physiological and pathological conditions [33]. In addition, mitochondria are largely responsible for reactive oxygen species (ROS) production, thereby resulting in a predisposition to oxidative stress [34]. An excessive level of oxidative stress disrupts mitochondrial membrane integrity which will lead to cardiac mitochondrial membrane depolarization and opening of the mitochondrial permeability transition pores (mPTP), followed by mitochondrial swelling [33]. Cytochrome *c* is released from the mitochondrial intermembrane space to form an apoptosome, which ultimately induces apoptosis [33]. Since cardiac mitochondrial dysfunction was found in cases of both testosterone deprivation and cardiac I/R [15, 17, 35], the severity of cardiac mitochondrial dysfunction was potentially increased in testosterone deprived rats with cardiac I/R [35].

There is only one *ex vivo* study which reported that testosterone deprivation for 4 weeks did not alter LV function and apoptosis in rats [27]. However, myocardial infarct size and arrhythmia status were not determined in that study [27]. The findings from this *ex vivo* report were not consistent with the *in vivo* studies, indicating that the duration of testosterone deprivation as well as the study models could influence the effects of testosterone on the heart.

Although testosterone deprivation has been reported to cause adverse effects on the heart with I/R, inconsistent reports exist. Two studies have reported that myocardial infarct size was decreased in testosterone deprived rodents [24, 28]. Myocardial infarct size was decreased 3 weeks after ORX (in a rat model) [28], and 20 weeks after ORX (in a mouse model) [24]. To the contrary, other studies reported that testosterone deprivation increased myocardial infarct size in rats between 2 and 12 weeks after ORX [10, 21–23]. The discrepancy in myocardial infarct size might be due to differences in the duration of testosterone deprivation and cardiac I/R protocol. There is an inconsistency in the data in cases of short-term testosterone deprivation. Two weeks of testosterone deprivation increased myocardial infarct size in an *in vivo* setting after 60 min of ischemia and 4 h of reperfusion. On the other hand, 3 weeks of testosterone deprivation decreased myocardial infarct size in an *ex vivo* setting after 30 min of ischemia and 150 min of reperfusion. Thus, the difference in cardiac I/R setting such as ischemia and reperfusion duration might involve a discrepancy of myocardial infarct size in testosterone deprived rats with cardiac I/R. A comprehensive summary of these reports is shown in Table 3.1.

As regards LV function, in *ex vivo* studies testosterone deprivation for 7 weeks has been shown to have both deteriorating and beneficial effects [20, 25, 26, 29, 31, 32]. Testosterone deprived rats who were subjected to 20 min of ischemia resulted in

a worsening of LV dysfunction [20]. However, testosterone deprived rats subjected to 25 min of ischemia showed an improvement in LV function during cardiac I/R, all rats undergoing similar reperfusion duration (40 min) [26, 29, 31, 32]. These findings suggest that the ischemic duration could be a key factor in the mediation of LV function in testosterone deprived rats with cardiac I/R. It has been proposed that testosterone deprivation attenuated LV dysfunction during cardiac I/R by increasing the level of survival protein kinase, enhancing antioxidants, reducing apoptosis [26, 29, 32] and inflammation [31]. This finding was supported by another *in vivo* study in which a short duration of ischemia (5 min) and reperfusion (5 min) was shown to reduce LV dysfunction in testosterone deprived rats by promoting cardiac mitochondrial respiration [30]. A comprehensive summary of these reports is shown in Table 3.1.

Metabolic syndrome is associated with low testosterone levels [36]. It has been shown that the percentage of body fat has a negative correlation with circulating testosterone level [36]. In obese—insulin resistant rats, testosterone deprivation did not increase myocardial infarct size, or arrhythmias, and it did not alter intracellular molecular signaling, compared with their sham operated rats [10, 37]. However, a worsening of LV dysfunction was reported in one study in which it was shown that testosterone deprivation increased LVEDP and left ventricular end systolic pressure (LVESP) in obese—insulin resistant rats, when compared with their sham operated controls [10]. These data indicated that, in a rodent model of metabolic syndrome, although testosterone deprivation did not increase myocardial infarct size, it aggravated LV dysfunction. A comprehensive summary of these reports is shown in Table 3.1.

The Effects of Exogenous Testosterone Administration on the Heart with Cardiac I/R

Testosterone acts via two major pathways: the genomic and the non—genomic pathways [5]. In the genomic pathway, after testosterone enters the cytosol it can be converted to dihydrotestosterone (DHT) by an enzyme 5α —reductase [5]. DHT then binds with an androgen receptor (AR), which forms a homodimer and undergoes a conformational change [5]. This AR complex then binds with other proteins to facilitate their nuclear translocation [5]. After the AR complex enters the nucleus, it binds to the androgen response elements (ARE), which are found on several promoter sites on the DNA [5]. ARA 70, a coactivator, is subsequently recruited for the transcription of AR-regulated genes, which can exert their action on target organs via their protein products [5]. In the non-genomic pathway, this process requires neither AR nuclear translocation nor AR-DNA-binding. The activated AR in the cytoplasm interacts with other signaling pathways such as PI3K/Akt, Ras/Raf and Src [5]. These signaling molecules activate the mitogen-activated protein kinase (MAPK)/extracellular signal—regulated kinase (ERK) pathway [5]. The activation

of the MAPK/ERK pathway is associated with cell proliferation gene expression [5]. In this chapter, we have summarized the effects of exogenous testosterone administration on the heart during cardiac I/R in animals with normal plasma testosterone levels.

In an *in vitro* study, the isolated cardiomyocytes were subjected to 30 min of ischemia (by incubating the cells with an ischemic solution) and 30 min of reperfusion (by incubating the cells with normal culture media) [38]. Testosterone was added to the cells during ischemia until the end of reperfusion. Cellular electrophysiological studies were performed, and the results showed that testosterone reduced action potential duration (APD₉₀) and the number of premature ventricular contractions (PVC) [38]. However, the resting membrane potential, the action potential amplitude, and V_{max} were not affected by the application of testosterone [38]. These findings suggested that testosterone could reduce arrhythmias at the cellular level. A comprehensive summary of these reports is shown in Table 3.2.

In the case of *ex vivo* studies, pretreatment with a very low dose of testosterone (0.001 nM) in isolated rat hearts was not shown to confer any level of protection on the heart regarding cardiac I/R injury [39]. Pretreatment with testosterone at a dose of 3 μg/kg improved LV function, but it did not reduce LDH levels after cardiac I/R [40]. In addition, pretreatment with a high dose of testosterone (5 mg/kg) increased myocardial infarct size by decreasing antioxidant levels, but it did not alter the level of apoptosis [41]. These data suggested that, in rats with a normal circulating testosterone level, the effects of exogenous testosterone were dependent on the dosage of exogenous testosterone. A summary of these reports is shown in Table 3.2.

The Effects of Exogenous Testosterone Administration on the Hearts of Testosterone Deprived Rats with Cardiac I/R

In one *ex vivo* study, testosterone deprived animals were given exogenous testosterone prior to cardiac I/R in a range of dosages and for various durations of treatment. The study reported that pretreatment with a single dose of testosterone injection (intraperitoneal injection; IP) at 10 mg/kg 6 h prior to cardiac I/R did not reduce LV dysfunction in rats and apoptosis was increased [27]. Another *ex vivo* study showed that pretreatment with a single dose of testosterone injection (intramuscular injection; IM) at 500 mg/kg, 14 days prior to cardiac I/R significantly attenuated LV dysfunction by reducing intracellular calcium levels, possibly through an increase in sarcoplasmic reticulum calcium ATPase levels [20]. This showed that a single high-dose of exogenous testosterone administration could protect the heart against cardiac I/R injury in testosterone deprived rats. A comprehensive summary of these reports is shown in Table 3.3.

Table 3.2 The effects of exogenous testosterone administration on a heart with cardiac I/R, in comparison with vehicle group

Animal models	I/R protocol	Testosterone regimen (Dose/Route/Duration)	Results			Molecular findings	Interpretation	Refs.
			Infarct size	LV function	Arrhythmias			
Isolated cardiomyocytes from rabbits	In vitro Simulated ischemic buffer 30 min/30 min	- 10, 100 nmol/L - During ischemia until end of reperfusion	N/A	N/A	- ↔ RMP, APA, Vmax - ↓ APD90, PVC	N/A	Testosterone administration reduced cellular electrophysiological changes in cardiomyocytes with I/R	[38]
Male Wistar rats	Ex vivo Global ischemia 30 min/30 min	- 0.001 nM - Mixed with perfusate - Pretreatment	↔	- ↔ Perfusion pressure, Coronary Resistance	N/A	N/A	Single testosterone administration prior to cardiac I/R did not alter LV function in rats with cardiac I/R	[39]
Male Wistar rats	Ex vivo Global ischemia 30 min/60 min	- 5 mg/kg - IM - 30 days - Pretreatment	↑	- ↑ LVEDP - ↔ LVSP - ↓ LVDP, ± dp/dt	N/A	- ↔ Bax, Bcl2, Bad SOD1,2,3 GPX1,3 - ↓ Catalase	Chronic pretreatment with testosterone aggravated LV dysfunction by decreasing antioxidant enzymes in rats with cardiac I/R	[41]
Male Wistar rats	Ex vivo Global ischemia 40 min/40 min	- 3 µg/kg - SC - 10 days - Pretreatment	N/A	- ↑ Coronary flow - ↔ SBP, DBP	N/A	- ↔ LDH	Chronic pretreatment with testosterone increased coronary flow, but it did not alter cardiac injury marker in rats with cardiac I/R	[40]

I/R: ischemia/reperfusion; ORX: orchietomy; IM: intramuscular injection; SC: subcutaneous injection; LV: left ventricular; LVEDP: left ventricular end-diastolic pressure; LVSP: left ventricular systolic pressure; LVDP: left ventricular developed pressure; SBP: systolic blood pressure; DBP: diastolic blood pressure; SOD: superoxide dismutase; GPX: glutathione peroxidase; LDH: lactate dehydrogenase; N/A: not assessed

Table 3.3 The effects of exogenous testosterone administration on the heart from testosterone deprived animals with cardiac I/R, in comparison with vehicle group

Animal models	I/R protocol	Testosterone regimen (Dose/Route/Duration)	Results		LV function	Arrhythmias	Molecular findings	Interpretation	Refs
			Infarct size	LV function					
ORX rats	Ex vivo Global ischemia 20 min/30 min	-10 mg/kg -IP -6 h prior to I/R -Pretreatment	N/A	-↔ LVDP	N/A	-↑ TUNEL ⁺ cells	Pretreatment with a single dose of testosterone did not alter LV function, but it increased apoptosis in testosterone deprived rats with cardiac I/R	[27]	
ORX rats	Ex vivo Global ischemia 25 min/40 min	-500 mg/kg -Single dose, IM -14 days prior to I/R -Pretreatment	N/A	-↑ LVDP -↓ LVEDP	N/A	-↓ Intracellular Ca ²⁺	Pretreatment with a single dose of testosterone improved LV function by decreasing intracellular calcium levels in testosterone deprived rats with cardiac I/R	[20]	
ORX rats	Ex vivo Global ischemia 25 min/40 min	-100 mg -Subcutaneous implantation -3 weeks -Pretreatment	N/A	-↔ ± dP/dt, LVDP	N/A	-↔ p-Akt/Akt, p-Bad, Bcl2, Bim, Fasl, MnSOD -↓ Bax, p-STAT3/STAT3	Chronic pretreatment with testosterone did not alter LV function, but it reduced apoptosis in testosterone deprived rats with cardiac I/R	[26, 32]	
ORX rats	Ex vivo Global ischemia 20 min/45 min	-100 mg/day -Subcutaneous implantation -3 weeks -Pretreatment	N/A	-↑ Aortic flow, CO, Cardiac work, LVDP, + dP/dt -↔ Coronary flow, -dP/dt, HR, SBP, DBP	N/A	-↑ Catalase -↔ MnSOD, GPX -↓ LDH	Chronic pretreatment with testosterone improved LV function by increasing antioxidant in testosterone deprived rats with cardiac I/R	[19]	

(continued)

Table 3.3 (continued)

Animal models	I/R protocol	Testosterone regimen (Dose/Route/Duration)	Results				Interpretation	Refs
			Infarct size	LV function	Arrhythmias	Molecular findings		
ORX rats	Ex vivo LAD ligation 30 min/120 min	-2 mg/kg -SC -8 weeks -Pretreatment	↓	-↑ LVDP; ± dP/dt -↓ LVEDP	-↓ PVB, VT	-↓ LDH	Chronic pretreatment with testosterone reduced infarct size, improved LV function, and arrhythmias by decreasing cardiac damage in testosterone deprived rats with cardiac I/R	[23]
ORX rats	Ex vivo LAD ligation 30 min/120 min	-2 mg/kg -SC -8 weeks -Pretreatment	↓	-↑ LVDP; ± dP/dt -↔ HR, Coronary flow -↓ LVEDP	N/A	-↔ HSP70, HSF1 -↓ LDH	Chronic pretreatment with testosterone reduced infarct size and improved LV function by decreasing cardiac damage in testosterone deprived rats with cardiac I/R	[21]
ORX mice	In vivo LAD ligation 1 h/30 days	-346 ng/kg -SC -Every 72 h for 30 days -Pretreatment	↓	N/A	N/A	-↓ Cellular infiltration, MMP3	Chronic pretreatment with testosterone reduced infarct size by reducing inflammation in testosterone deprived rats with cardiac I/R	[42]
ORX rats	In vivo LAD ligation 30 min/120 min	-2 mg/kg -SC -4 weeks -Pretreatment	↓	-↑ SV -↓ LVEDP; -dP/dt	-↑ Time to 1stVT/VF onset -↓ Arrhythmia score	-↑ p-Cx43/Cx43, Procaspase 3 -↓ Bax/Bcl2, Mito ROS, Mito membrane depolarization, Mito swelling	Chronic pretreatment with testosterone reduced infarct size and arrhythmias, and improved LV function by increasing gap junction proteins, reducing apoptosis and cardiac mitochondrial dysfunction in testosterone deprived rats with cardiac I/R	[10, 35]
ORX rats	In vivo LAD ligation 60 min/4 h	-0.02, 0.2, 2.02 µg/kg -IV -During ischemia	↓	N/A	N/A	N/A	Single dose of testosterone administration during ischemia reduced infarct size in testosterone deprived rats with cardiac I/R	[22]

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Table 3.3 (continued)

Animal models	I/R protocol	Testosterone regimen (Dose/Route/Duration)	Results			Interpretation		Refs
			Infarct size	LV function	Arrhythmias	Molecular findings		
ORX Obese –insulin resistant rats	In vivo LAD ligation 30 min/120 min	-2 mg/kg -SC -4 weeks -Pretreatment	↓	-↑ SV -↓ LVEDP, -dP/dt	-↑ Time to 1st VT/VF onset -↓ Arrhythmia score	-↑ p-Cx43/Cx43, Procaspase 3 -↓ Bax/Bcl2, Mito ROS, Mito membrane depolarization, Mito swelling	Chronic pretreatment with testosterone reduced infarct size, arrhythmias, and improved LV function by increasing gap junction protein, reducing apoptosis and cardiac mitochondrial dysfunction in testosterone deprived obese—insulin resistant rats with cardiac I/R	[10]
ORX Obese –insulin resistant rats	In vivo LAD ligation 45 min/120 min	-2 mg/kg -Subcutaneous implantation -4 weeks -Pretreatment	↓	N/A	N/A	-↓ CKMB	Chronic pretreatment with testosterone reduced infarct size via reducing cardiac damage in testosterone deprived obese—insulin resistant rats with cardiac I/R	[37]
Naturally aged rats	Ex vivo Global ischemia 30 min/60 min	-1 mg/kg -SC -30 days -Pretreatment	↑	-↑ LVDP, ± dP/dt CI -↔ LVSP LVEDP	N/A	-↔ GSK3β, Caspase3, Bcl2, GRP78, CHOP, eIF2α -↓ p-Akt/Akt	Although chronic pretreatment with testosterone improved LV function, it increased infarct size in aged rats. These effects were mediated by a reduction of survival kinase, however, it did not alter apoptosis and ER stress	[43]

I/R: ischemia/reperfusion; ORX: orchectomy; IP: intraperitoneal injection; IM: intramuscular injection; SC: subcutaneous injection; LV: left ventricular; LVEDP: left ventricular end-diastolic pressure; LVESP: left ventricular end systolic pressure; LVSP: left ventricular systolic pressure; LVDP: left ventricular developed pressure; SV: stroke volume; CO: cardiac output; CI: cardiac index; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; PVB: premature ventricular beat; VT: ventricular tachycardia; VF: ventricular fibrillation; ROS: reactive oxygen species; Mito: mitochondria; HSP: heat shock protein; HSF: heat shock factor; LDH: lactate dehydrogenase; MMP: matrix metalloproteinase; Cx: connexin; MnSOD: manganese superoxide dismutase; GPX: glutathione peroxidase; LDH: lactate dehydrogenase; CKMB: creatine kinase MB isoenzyme; GSK3β: Glycogen synthase kinase 3 beta; GRP78: Glucose regulatory protein 78; CHOP: C/EBP homologous protein; eIF2α: Eukaryotic initiation factor 2 alpha; N/A: not assessed

As regards chronic treatment, testosterone was subcutaneously implanted in testosterone deprived rats (100 mg, 3 weeks) prior to cardiac I/R [19, 26, 32]. The results showed that, in the setting of ex vivo cardiac I/R (25 min ischemia and 40 min reperfusion), testosterone attenuated apoptosis but not LV dysfunction [26, 32]. Another study showed that, in an ex vivo setting of cardiac I/R (25 min ischemia and 45 min reperfusion), testosterone attenuated LV dysfunction and increased antioxidant enzyme level [19]. These findings suggested that, with similar doses of testosterone, the duration of reperfusion may affect LV function in testosterone deprived rats with cardiac I/R. A comprehensive summary of these reports is shown in Table 3.3.

The method of regional ischemia performed in some ex vivo studies was ligation of the LAD [21, 23]. Exogenous testosterone (2 mg/kg) was given to the testosterone deprived rats daily for 8 weeks prior to cardiac I/R, and the results demonstrated that testosterone could reduce myocardial infarct size and LV dysfunction [23]. In addition, the numbers of PVC and VT were decreased in testosterone deprived rats with cardiac I/R [23]. Another study showed that testosterone could reduce LDH but it did not affect the heat shock proteins HSP70 and HSF1 [21]. HSP70 and HSF1 are stress inducible proteins which are excessively expressed during cardiac I/R [21]. An activation of the HSP70–HSF1 complex could initiate apoptosis and an inflammatory response [21]. However, the beneficial effects of testosterone were not mediated by this protein. A comprehensive summary of these reports is shown in Table 3.3.

In *in vivo* studies, the effects of acute and chronic exogenous testosterone have been investigated in testosterone deprived rats. The acute administration of exogenous testosterone (0.2 and 2.0 $\mu\text{g}/\text{kg}$, IV), given to the testosterone deprived rats during cardiac ischemia was shown to reduce myocardial infarct size [22]. However, the precise molecular mechanism responsible for the beneficial effects in acute treatment was not determined [22]. In a chronic pretreatment model, exogenous testosterone (346 ng/kg and 2 mg/kg, 4 weeks) effectively reduced myocardial infarct size, LV dysfunction, and arrhythmias in testosterone deprived rats [10, 35, 42]. These studies proposed that testosterone could reduce cardiac inflammation [42], oxidative stress, apoptosis and cardiac mitochondrial dysfunction [10, 35] in testosterone deprived rats. These beneficial effects of exogenous testosterone were also observed in the obese–insulin resistant rats with testosterone deprivation subjected to cardiac I/R through a similar procedure [10]. A summary of these reports is shown in Table 3.3.

One study used a naturally aged rat model with age related low testosterone levels [43]. Exogenous testosterone was given to the rats at 1 mg/kg for 30 days prior to cardiac I/R, and the results showed that this intervention increased myocardial infarct size, when compared with the vehicle control group [43]. However, the degree of LV dysfunction was decreased. The molecular mechanism demonstrated that exogenous testosterone decreased p-Akt/Akt, which is a survival protein, however it did not affect apoptosis and ER stress levels [43] (Table 3.3). These data suggested that exogenous testosterone reduced LV dysfunction through the activation of this survival protein.

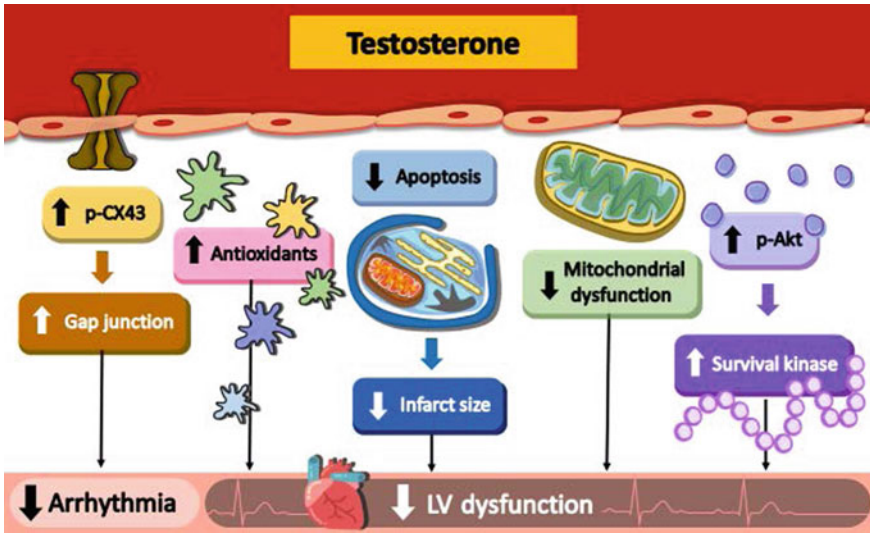


Fig. 3.1 This summary diagram demonstrates the potential mechanisms involved in the mediation of the action of testosterone during cardiac ischemia/reperfusion including the activation of Cx43, antioxidants, apoptosis, mitochondrial function, and survival kinase. Cx43: connexin 43; LV: left ventricle

From these reports, it could be concluded that exogenous testosterone application reduced cardiac mitochondrial dysfunction and led to a reduction in cardiac apoptosis, resulting in a reduction in myocardial infarct size and LV dysfunction. In addition, exogenous testosterone reduced matrix metalloproteinase activity, cellular infiltration, and inflammation in testosterone deprived rats. A summary regarding the roles of testosterone on the heart of cardiac I/R is depicted in Fig. 3.1.

The Effects of Testosterone Replacement Therapy (TRT) on Cardiovascular Outcome in Clinical Reports

A previous study has shown that testosterone plays an important role in regulating cardiac damage during MI, specifically that testosterone deprivation led to an increased incidence of microvascular obstruction in STEMI patients [7]. Therefore, TRT is used to prevent cardiac complications in patients with low circulating testosterone levels. There are large cohort studies showing that long-term TRT use (for more than 17 months) could increase cardiovascular event free survival rate, decrease all-cause mortality, and reduce major cardiac adverse events in middle to old aged men with low testosterone levels [44, 45, 47, 48]. However, TRT for 1 year has been shown neither to increase nor decrease MI events in middle to old aged men with low testosterone levels [46]. Although long-term TRT showed benefits

as regards the cardiovascular outcomes, two studies have reported adverse effects of TRT in increasing the incidence of obstructive sleep apnea [44] and stroke [48]. In older aged men (the age of all participants was greater than 70 years old), TRT promoted MI risk by increasing noncalcified coronary artery plaque volume, total plaque volume, and coronary artery calcium score [49]. These findings suggested that TRT provided several benefits on cardiovascular outcomes in the middle to old aged male cohort who have low testosterone levels, however, testosterone prescription to the older aged men (subjects over 70 years old) might increase risk for MI. A comprehensive summary of these reports is shown in Table 3.4.

Previous studies also reported the effects of TRT on cardiovascular outcomes in men with low testosterone levels, together with MI or heart failure. In the case of both short-term (1 month) and longer-term (3 months–1 year) treatment, TRT reduced cardiac adverse events by delaying the time to ischemia [50] and time to 1 mm ST segment depression [51], reducing carotid intima–media thickness [50] and QT dispersion [52] in men with low testosterone and heart disease. However, long–term TRT did not affect MI events in aging men (subjects over 70 years old) with MI and low testosterone levels [53]. This information indicated that TRT reduced cardiac adverse events in middle to old aged men who have cardiac complications and low testosterone levels. A summary of these reports is shown in Table 3.4.

Table 3.4 Clinical reports concerning the effects of exogenous testosterone administration on cardiovascular outcomes

Study design	TRT regimen	Results	Interpretation	Refs
Men with low testosterone levels (0–60 years)	TRT group (n = 3,422) versus Control (n = 3,422) F/U = 17 month	–↑ CV event free survival –↓ CAD event –↔ CHF event Stroke event –↑ OSA	TRT increased survival rate and reduced risk of coronary artery disease, but it did not affect chronic heart failure and stroke event in men with low testosterone levels. However, TRT increased obstructive sleep apnea event	[44]
Men with low testosterone levels (Age ≥ 50 years)	TRT group (n = 8,137) versus Control (n = 4,418) Mean F/U = 6.6 years	↓ CHF event CAD event All–cause mortality MI event	TRT reduced mortality and cardiovascular events in men with low testosterone levels	[45]
Men with low testosterone levels (Age > 50 years)	TRT group (n = 207,176) versus Control (n = 207,176) F/U = 1 year	↔ MI event	TRT did not affect MI events in men with low testosterone levels	[46]

(continued)

Table 3.4 (continued)

Study design	TRT regimen	Results	Interpretation	Refs
Men with low testosterone levels (Age \geq 50 years)	TRT group (n = 43,931) versus Control (n = 13,378) Mean F/U = 6.2 years	↓ All-cause mortality MI event Stroke event	TRT reduced mortality and cardiovascular events in men with low testosterone levels	[47]
Men with low testosterone levels (Age > 60 years)	TRT group (n = 2,241) versus low testosterone group (n = 801) F/U = 3 years	↓ MACE ↑ Stroke event	TRT reduced major cardiac adverse events, but it increased stroke events in men with low testosterone levels	[48]
Aging men with low testosterone levels (Age > 70 years)	TRT group (n = 73) versus Control (n = 65) F/U = 1 year	↑ non calcified coronary artery plaque volume Total plaque volume Coronary artery calcium score	TRT increased coronary plaques in aging men with low testosterone levels	[49]
Men with stable, chronic angina pectoris and low testosterone levels (Age > 20 years)	TRT group (n = 7) versus Control (n = 6) F/U = 1 year	↑ Time to ischemia ↓ Carotid intima-media thickness	TRT delayed time to ischemia and reduced carotid intima-media thickness in men with stable chronic angina pectoris and low testosterone levels	[50]
Men with ischemic heart disease and low testosterone levels (Age > 60 years)	TRT group (n = 11) versus Control (n = 10) F/U = 1 month	↑ Time to 1 mm ST segment depression	TRT delayed time to ischemia in men with ischemic heart disease and low testosterone levels	[51]
Men with heart failure and low testosterone levels (Age > 60 years)	TRT group (n = 44) versus their baseline F/U = 3 month	↓ QTd	TRT reduced QT dispersion in men with heart failure and low testosterone levels	[52]
Aging men with MI and low testosterone levels (Age > 70 years)	TRT group (n = 299) versus non-TRT group (n = 29,551) Mean F/U = 2.8 years	↔ MI events	TRT did not alter incidence of MI events in aging men with MI and low testosterone levels	[53]

TRT: testosterone replacement therapy; F/U: follow-up; MI: myocardial infarction; CV: cardiovascular; CAD: coronary artery disease; CHF: chronic heart failure; OSA: obstructive sleep apnea; MACE: major adverse cardiac event

Conclusion

Testosterone plays an important role in the regulation of myocardial injury during cardiac I/R, the extent of the impact being dependent on the duration of testosterone deprivation. Exogenous testosterone administration modulates myocardial infarction, LV function, and arrhythmias during cardiac I/R injury, the degree of modulation being dependent on dose and time of administration in both normal and testosterone deprived rats.

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