

# Testosterone and the Cardiovascular System: A Comprehensive Review of the Basic Science Literature

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An increasing number of elderly men are receiving exogenous testosterone to treat hypogonadism, low libido, and general weakness.<sup>1</sup> The impact of testosterone on the cardiovascular system is controversial. Elderly men are typically at higher risk for adverse cardiovascular events than age-matched women, and one study suggested that exogenous testosterone was associated with an increase in adverse cardiovascular events in this population.<sup>2</sup> In contrast, other clinical studies suggest that testosterone is beneficial to the cardiovascular system and that low levels of testosterone negatively affect the cardiovascular system.<sup>3,4</sup>

Because of this controversy, we sought to determine the current status of basic science studies that have examined the effects of testosterone on the cardiovascular system in experimental models.

## Action Potential Duration, Early After Depolarization, and QTc

Testosterone may have antiarrhythmic properties at high enough concentrations. Pham et al tested dofetilide, an antiarrhythmic agent that also may have proarrhythmic properties, against varying levels of testosterone in ventricular myocytes.<sup>5</sup> The concentration of testosterone was measured against 90% action potential duration (APD<sub>90</sub>) and percent incidence of early after depolarizations (EADs). A longer APD<sub>90</sub> and a higher incidence of EADs suggest a higher propensity toward arrhythmia. In this study, dofetilide was given to different groups of rabbits: normal males, normal females,

orchiectomized males, dihydrotestosterone (DHT)-treated orchiectomized males, and DHT-treated females. DHT was given in the form of a 60-day extended-release pellet implanted in the animal. The papillary muscle was removed and placed in a physiological solution; then action potential time was measured in vitro. Pham et al found that male and female groups with testosterone had both shorter APD<sub>90</sub> and a decreased percentage in the incidence of EADs.<sup>5</sup> These findings suggest a possible antiarrhythmic property of testosterone.

Bai et al<sup>6</sup> arrived at similar conclusions. Researchers in this study investigated single ventricular myocytes isolated from guinea pig hearts that were placed in a physiological solution. As the myocytes were exposed to higher concentrations of testosterone, APD<sub>20</sub> and APD<sub>90</sub> both decreased, providing further evidence to support the antiarrhythmic effects of testosterone.<sup>6</sup>

Brouillette et al<sup>7</sup> examined the effects of androgens on ventricular repolarization in genetically low-testosterone mice and mice with genetically normal physiological levels of testosterone. Male and female mice were included in this study. Researchers hypothesized that androgen-deficient mice (testosterone or DHT) would exhibit ventricular repolarization characteristics similar to female mice, whereas mice with physiological levels of testosterone, achieved endogenously or by supplementation, would exhibit comparatively shorter ventricular repolarization times. Ventricular myocytes were examined in vitro using whole-cell voltage and current clamps. Orchiectomized males treated with testosterone demonstrated shortened action potential duration as well as shortened QTc interval compared with females and testosterone-deficient males. In addition, Western blots were performed to determine Kv1.5 K<sup>+</sup> channel expression, which is responsible for I<sub>KUR</sub>, the ultra-rapid delayed K<sup>+</sup> rectifier.<sup>7-9</sup> Western blots uncovered increased Kv1.5 K<sup>+</sup> channel expression and increased I<sub>KUR</sub> in testosterone-normal males when compared with females and testosterone-deficient males. Therefore, testosterone-shortened ventricular repolarization duration and the mechanism of testosterone may have involved increasing expression of the Kv1.5 K<sup>+</sup> channels.<sup>7-9</sup>

The experimental literature shows that testosterone causes beneficial cardiovascular effects with regard to APD and EADs and decreases QTc interval length. Whether by increasing

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K<sup>+</sup> channel expression to better stabilize the cardiomyocyte<sup>7</sup> or as a yet unexamined or undiscovered mechanism, testosterone may create an antiarrhythmic substrate.

## Cardioprotection

Testosterone may contribute to the reduction of myocardial infarction (MI) size in rats. In a study by Liu et al,<sup>8</sup> male rats were subjected to an orchiectomy or a sham orchiectomy. Orchiectomized rats were given either testosterone treatment or served as controls. Testosterone dosing was 2 mg of testosterone per kilogram of body weight daily for 8 weeks, which approximates physiological levels. Rats in each group were either preconditioned with U50 488H, a  $\kappa$ -opioid receptor agonist, or not preconditioned. MI size in the orchiectomy group was significantly larger than both the sham and orchiectomy plus testosterone groups. Infarct size in the orchiectomy plus testosterone group closely resembled the sham group. The sham plus preconditioning group had a significantly smaller MI size than did the sham group. This finding was repeated in the orchiectomy and the orchiectomy plus testosterone groups, but reached statistical significance only when comparing the orchiectomy plus testosterone group with the sham group.<sup>8</sup>

Tsang et al provided support for the findings by Liu and coworkers.<sup>9</sup> Isolated rat hearts were subjected to coronary artery occlusion (regional ischemia) for 30 minutes, followed by 120 minutes of reperfusion with a physiological solution. Rats used were control, orchiectomized, or orchiectomized plus testosterone supplementation. After testosterone was given, at 2 mg/kg body weight, daily for 8 weeks starting 1 week after orchiectomy, hearts were isolated. Hearts of orchiectomized rats had a significantly larger MI size than did the control group. The MI size of the orchiectomy group was significantly larger than that of the orchiectomy plus testosterone group,<sup>10</sup> again suggesting a cardioprotective effect of testosterone.

A supporting study by Liu et al<sup>11</sup> studied the effect of testosterone on estradiol's cardioprotective potential in isolated ventricular myocytes from ovariectomized or sham-ovariectomized female rats. It has been well established that estrogen benefits the cardiovascular system by reducing myocardial injury after ischemia and reperfusion,<sup>12–15</sup> but few studies have examined the effects of estrogen in the presence of testosterone on the cardiovascular system. Rat ventricular myocytes were subjected to 3 hours of ischemia by incubation in an ischemic buffer in hypoxic conditions (1% oxygen), followed by 4 hours of reperfusion by replacing the ischemic buffer with a physiological solution at normal atmosphere.<sup>11</sup> The results of this study demonstrated that both testosterone supplementation and testosterone plus estrogen supplementen-

tation in cardiomyocytes of ovariectomized rats reduced apoptosis occurring as a result of ischemia plus reperfusion. Hormone supplementation also improved contractility compared with ovariectomized rat myocytes without testosterone or estrogen supplementation.<sup>11</sup>

Er et al<sup>16</sup> proposed a mechanism through which testosterone exerts a positive effect on the cardiovascular system. The authors hypothesized that mitochondrial K<sub>ATP</sub> channels play an important role in cardioprotection. These researchers examined the changes in myocardial tolerance to ischemia with varying mitochondrial or sarcoplasmic K<sub>ATP</sub> channel efficacy. To accomplish this, Er and coworkers isolated ventricular cardiomyocytes from rats. To simulate ischemia, cardiomyocytes were centrifuged into a pellet and had mineral oil applied to prevent gas exchange for 60 minutes. In some experiments, mitochondrial or sarcoplasmic K<sub>ATP</sub> channel inhibitors were added. Results of this study showed that testosterone decreased the percentage of dead cells per sample by approximately 50%. Testosterone also played a role in acutely oxidizing and depolarizing cardiac mitochondria. This was accomplished by a K<sup>+</sup>-dependent, ATP-sensitive mechanism, which was independent of mitochondrial androgen receptors (no difference between the testosterone and testosterone plus flutamide groups). Testosterone's beneficial effect on cell death was mitigated by 5-hydroxydecanoate, a mitochondrial K<sub>ATP</sub> channel inhibitor, although 5-HD treatment alone had no effect on cell death compared with the control. Testosterone had no effect on sarcoplasmic K<sub>ATP</sub> channels. These results demonstrated a positive cardioprotective effect of testosterone that may involve opening the mitochondrial K<sub>ATP</sub> channels, allowing K<sup>+</sup> to more efficiently exit the mitochondria, resulting in increased stability of the mitochondria.<sup>16</sup> Other mechanisms of cardioprotective effects of testosterone including vasodilation, lipid formation inhibition, and atherosclerotic inhibition are discussed later.

Basic science studies suggest that testosterone protects the heart from ischemic injury. There is little information regarding the mechanism by which testosterone exerts its cardioprotective effect regarding ischemic injury; thus, more research is required.

## Vasodilation

There is growing evidence suggesting that testosterone has a vasodilatory effect on blood vessels. Yue et al<sup>17</sup> examined the coronary arteries of rabbits with and without the endothelium. The vasodilator nitric oxide (NO) is synthesized in the endothelium. Effects of a therapy on blood vessels that have been subjected to endothelial denudation would suggest that the drug is working through an endothelium-independent and NO-mediated-independent mechanism, such as directly on

the tunica media (smooth muscle layer) of an artery. Yue et al exposed arteries to prostaglandin to contract the tunica media. After 7 minutes in prostaglandin, arteries were washed and exposed to testosterone or control solution. The relaxation percentage, which is the amount of relaxation induced by testosterone compared with the contraction induced by prostaglandin, was determined. Testosterone concentration and relaxation percentage increased concurrently. Statistical significance was observed at both 1 and 10  $\mu\text{mol/L}$  of testosterone, and there was no difference between the groups with and without endothelium.<sup>16</sup> This suggests that testosterone has a direct smooth muscle-relaxing effect and does not require endothelium to induce vasodilation.

Deenadayalu et al<sup>18</sup> performed a similar study using the left anterior descending (LAD) coronary arteries of swine hearts. Again, prostaglandin was used as a contractile agent. This study tested the effect of endothelial denudation as well as washing the vessels with either Krebs–Henseleit bicarbonate (KHB), or N-nitro-L-arginine methyl ester (L-NAME). KHB is a physiological buffer, whereas L-NAME is a compound that inhibits the production of nitric oxide synthase (NOS), thus inhibiting NO from entering the vessel. An increase in testosterone concentration yielded an increase in relaxation percentage compared with the controls, providing further evidence that testosterone causes vasodilation. Endothelial denudation and L-NAME treatment had no effect on this result.<sup>18</sup> This finding is directly supported by a study done by Tep-areenan et al<sup>19</sup> in rat arteries.

Crews and Kahill<sup>20</sup> compared the initial degree of maximum contraction of endothelial-denuded, extracted LAD arterial segments of rats to the percentage of contraction of rat LAD segments that had been treated with testosterone. The LAD was isolated and placed in either prostaglandin or potassium chloride (KCl), another contracting agent, and testosterone. At testosterone concentrations between  $10^{-5}$  and  $10^{-4}$  mol/L, the vessels treated with KCl showed greater contraction than those contracted with prostaglandin, but the trend of reduced contraction with increased testosterone concentration was still seen, suggesting that testosterone may be a vasodilator.<sup>20</sup>

In a study examining the effect of acute testosterone administration on the diameter of the microvasculature, O'Connor et al<sup>21</sup> isolated and removed the coronary arterioles of male, nonorchietomized pigs after administering physiologic levels of testosterone, 5 $\alpha$ -dihydrotestosterone, or epitestosterone, a testosterone epimer previously believed to be biologically inactive, into the coronary arteries. Some animals received flutamide. In vivo, coronary arterioles were examined using a Transonic perivascular flow meter, which is a device that is used to measure blood flow. Testosterone and 5 $\alpha$ -dihydrotestosterone administration yielded increased cor-

onary blood flow independent of dose in intact animals, suggesting that vessels were dilated. This was not seen in castrated animals or intact animals subjected to epitestosterone administration. A strong trend existed suggesting flutamide diminished the increase in flow seen in animals treated with 5 $\alpha$ -dihydrotestosterone, suggesting that the androgen receptor does affect this mechanism when 5 $\alpha$ -dihydrotestosterone is used. This trend was not seen in the epitestosterone treatment group. Vessels of intact animals dilated when given either of the 3 testosterone treatments without flutamide were compared with baseline. Vasodilation increased as testosterone concentration increased. This was also seen in orchietomized animals except in the epitestosterone group. There was no difference between the groups in androgen receptor expression.<sup>21</sup> This was the only study to suggest that flutamide attenuates the vasodilatory effect of testosterone in models using 5 $\alpha$ -dihydrotestosterone. Although the data did not reach statistical significance, this trend should not be ignored when attempting to elucidate the vasodilatory mechanism of testosterone.

Jones et al<sup>22</sup> extracted the coronary arteries and thoracic aortas from male rats and placed them in a physiological buffer. Vessels had either a denuded endothelium, flutamide or KCl treatment, or were controls. Before treatment, each vessel was incubated in prostaglandin-F (PGF), a contractile agent, before treatment until maximum contraction had been achieved. Testosterone was then administered at concentrations between 0.1 and 100  $\mu\text{mol/L}$ . There was no difference between the relaxation percentages of vessels with and without an endothelium at different testosterone concentrations. Relaxation percentages of control vessels and vessels treated with flutamide did not differ either. There was no difference in the concentration-response curve of testosterone in vessels precontracted with KCl instead of PGF.<sup>22</sup> The ineffectiveness of endothelial denudation and flutamide incubation on relaxation percentages suggests that testosterone's vasodilatory effect is independent of both the endothelium and androgen receptors.

Much evidence for the vasodilatory effects of testosterone exists, but less is known about the mechanism through which testosterone causes vasodilation. Scragg et al<sup>23</sup> examined the possibility that testosterone is an inhibitor of voltage-gated calcium ( $\text{Ca}^{2+}$ ) channels. The authors examined smooth muscle cells from rats using whole-cell patch-clamps to record electrical currents. Increased concentrations of testosterone yielded a decrease in the testosterone to control current ratio ( $I[\text{test}]/I[\text{control}]$ ) in L-type  $\text{Ca}^{2+}$  at  $10^{-7}$  mol/L, or approximately physiological levels. This suggests that testosterone inhibits the L-type  $\text{Ca}^{2+}$  channel. An inhibition of T-type  $\text{Ca}^{2+}$  channels was also seen, but only at supraphysiological testosterone levels.<sup>23</sup> Thus, testosterone may have further vasodilatory effects at supraphysiological levels.

The aforementioned studies suggest that testosterone has a vasodilatory effect on arteries independent of the endothelium, suggesting that this vasodilation is not solely dependent on NO.<sup>17–22</sup> Studies also suggest that testosterone directly affects the tunica media of the arteries, possibly by inhibiting L-type and T-type calcium channels.<sup>23</sup> Much of this information can be viewed in Table 1.

## Lipids

A main risk factor for cardiovascular disease is metabolic syndrome (MetS). MetS encompasses hyperglycemia, hypertension, dyslipidemia, and central obesity.<sup>24</sup> Filipi et al<sup>24</sup> investigated the relationship between testosterone and MetS. Male rabbits with or without testosterone supplementation were fed a high-fat diet. These experimental rabbits were compared with control rabbits given a standard diet. Rabbits fed the high-fat diet displayed all the characteristics of MetS. Experimental rabbits also had decreased testicular and seminal vesicle weight, decreased testosterone levels, and reduced expression of enzymes necessary for testosterone synthesis (StAR, CYP17A1, and 3 $\beta$ -HSD) compared with controls. Testosterone supplementation reduced visceral fat accumulation, improved fasting glucose levels, glucose tolerance, and mean arterial pressure, while having no statistically significant impact on total cholesterol or triglyceride levels. When exogenous testosterone administration was stopped for 2 weeks, an increase in insulin resistance, an

important contributor to type 2 diabetes mellitus, was seen. This study showed that testosterone supplementation may reduce the MetS phenotype, thereby reducing the risk for cardiovascular disease.<sup>24</sup>

Gupta et al<sup>25</sup> examined the differentiation and proliferation of human mesenchymal stem cells (hMSCs) and preadipocytes in vitro in the presence of DHT. DHT inhibited the differentiation of hMSCs into adipocytes, as well as lipid accumulation in existing adipocytes. DHT also inhibited the maturation of preadipocytes into mature adipocytes. This was secondary to the downregulation of the adipogenic differentiation markers aP2, PPAR $\gamma$ , and C/EBP $\alpha$ . No difference was found in hMSC or preadipocyte proliferation in the presence of DHT, suggesting that DHT only inhibits the uptake of lipids into adipocytes and not other hMSC differentiation.<sup>25</sup>

These studies suggest that testosterone decreases total fat mass, therefore increasing net lean mass, and consequently may result in a stronger, more efficient body, which may be why testosterone is such an appealing performance-enhancing drug to athletes.<sup>24,25</sup> The aforementioned studies also suggest that men with higher endogenous levels of testosterone have greater lean body mass and lower body fat percentage than men with lower testosterone levels, and thus testosterone gels and patches are increasingly popular and widespread treatments in the field of antiaging,<sup>1</sup> as testosterone has been shown to increase physical performance, strength, and lean body mass in men suffering from low testosterone.<sup>26</sup>

**Table 1.** Effects of Testosterone on Vasodilation

Study	Model	End Points Measured	Major Findings
Deenadayalu et al <sup>18</sup>	Swine LAD	<ul style="list-style-type: none"> <li>Vessel relaxation percentage in the presence/absence of testosterone, endothelium, and NO inhibitors.</li> </ul>	<ul style="list-style-type: none"> <li>Increased testosterone concentrations resulted in increased relaxation percentages versus control.</li> <li>These effects were seen regardless of endothelial denudation or L-NAME incubation.</li> </ul>
Tep-areenan et al <sup>19</sup>	Rat LAD	<ul style="list-style-type: none"> <li>Vessel relaxation percentage in the presence/absence of testosterone, endothelium, and NO inhibitors.</li> </ul>	<ul style="list-style-type: none"> <li>Increased testosterone concentrations resulted in increased relaxation percentages versus control.</li> <li>These effects were seen regardless of endothelial denudation or L-NAME incubation.</li> </ul>
Crews and Kahill <sup>20</sup>	Rat LAD	<ul style="list-style-type: none"> <li>Vessel contraction with prostaglandin or KCl in the presence/absence of testosterone.</li> </ul>	<ul style="list-style-type: none"> <li>There was an inverse relationship between vessel contraction and testosterone concentration.</li> </ul>
O'Connor et al <sup>21</sup>	Swine coronary arteries	<ul style="list-style-type: none"> <li>Coronary conductance (relaxation) and vessel diameter in the presence/absence of testosterone and flutamide.</li> </ul>	<ul style="list-style-type: none"> <li>Increased coronary conductance in testosterone groups.</li> <li>Increased vessel diameter in testosterone groups.</li> <li>Flutamide had no effect.</li> </ul>
Jones et al <sup>22</sup>	Rat coronary arteries and thoracic aortas	<ul style="list-style-type: none"> <li>Relaxation percentages in the presence/absence of testosterone, endothelial denudation, and flutamide.</li> </ul>	<ul style="list-style-type: none"> <li>Increased testosterone concentrations resulted in increased relaxation percentages versus control.</li> <li>These effects were seen regardless of endothelial denudation or L-NAME incubation.</li> </ul>

LAD indicates left anterior descending; NO, nitric oxide; L-NAME, nitro-L-arginine methyl ester; KCl, potassium chloride.

## Diabetes Mellitus

The relationship between low testosterone levels and diabetes mellitus (DM) may not depend on testosterone; it may be a result of poorly controlled DM. In a study by Jackson and Hutson, diabetes was chemically induced in rats of both sexes.<sup>27</sup> Diabetic rats showed significantly higher blood glucose levels accompanied by statistically lower levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), insulin, and testosterone than control rats. Male diabetic rats also had lower seminal vesicle and ventral prostate gland weight compared with normal rats. Insulin administration, mimicking properly controlled DM, restored LH, FSH, and testosterone levels to normal, suggesting that testosterone levels alone have no effect on DM and that the control of DM may have an effect on testosterone levels.<sup>27</sup>

In a study by Ballester et al,<sup>28</sup> streptozotocin-diabetic rats were treated with sodium tungstate, a drug that normalizes blood glucose levels, to determine its effect on Leydig cell function. Sodium tungstate has not been shown to cause adverse effects such as the development of tolerance or acute hypoglycemic episodes.<sup>29</sup> The diabetic rats had significantly higher blood glucose levels and significantly lower LH, FSH, insulin, and testosterone levels than did the control rats.<sup>28</sup> Sodium tungstate administration normalized blood glucose, insulin, LH, FSH and testosterone concentrations. This suggests that poorly controlled DM may decrease testosterone levels, which suggests that low testosterone levels do not lead to diabetes,<sup>28</sup> confirming the previously described study.

## Atherosclerosis

Because the male sex is believed to be a risk factor for atherosclerosis, investigation of the relationship between testosterone and atherosclerosis has been explored.<sup>30</sup> Bruck et al<sup>31</sup> noticed differing results in the literature and thus tested the relationship between testosterone and atherosclerosis in a rabbit model. Orchiectomized male and ovariectomized female rabbits were fed a high-cholesterol diet for 12 weeks, and each sex was separated into 4 treatments of sex hormones: control, estradiol, testosterone, or estradiol plus testosterone. Atherosclerosis was examined in the proximal aortic arch by morphometric analysis. Estradiol treatment in the female rabbits and testosterone treatment in the male rabbits inhibited the buildup of atherosclerotic plaque compared with that in the control groups. The estradiol plus testosterone treatment inhibited plaque build up in both sexes compared with the control groups. This effect was not present in males treated with only estradiol and females treated with only testosterone, suggesting that when both hormones are present, the sex-specific hormone dominates

this mechanism and protects the vasculature from the buildup of atherosclerotic plaque.<sup>31</sup>

A similar in vitro study by Hanke et al<sup>32</sup> involved aortas from male rabbits fed standard chow. After euthanization, vessels were subjected to endothelial denudation using a balloon catheter, then extracted and cut into rings. These segments were then cultured for 21 days in a standard medium containing differing concentrations of testosterone. This study showed that testosterone inhibited neointimal plaque development at concentrations of 10 and 100 ng/mL. Testosterone also caused a 50% increase in the amount of androgen receptor mRNA. In this study, the testosterone level required to reduce atherosclerotic plaque was found to be near physiological levels.<sup>32</sup>

Alexandersen et al<sup>33</sup> found that testosterone inhibited atherosclerosis in a rabbit model. Male rabbits fed a high-cholesterol diet either underwent an orchiectomy or a sham orchiectomy. Orchiectomized rabbits were given either oral dehydroepiandrosterone (DHEA), oral testosterone undecanoate (TU), intramuscular testosterone enanthate (TE), or placebo. Orchiectomy resulted in a 90% increase in atherosclerotic plaque present compared with the sham group. The orchiectomy plus placebo group had much higher levels of atherosclerosis than all other groups, whereas the TE group had the lowest levels of atherosclerosis.<sup>33</sup> This study suggested that testosterone attenuates the formation of atherosclerotic plaque in this rabbit model.

Arad et al<sup>34</sup> examined DHEA versus placebo with regard to atherosclerosis in rabbits fed a high-cholesterol diet. DHEA increased very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and low-density lipoprotein (LDL) levels compared with levels before initiation of the high-cholesterol diet in tested rabbits. However, DHEA-treated rabbits had 40% less formation of fatty streaks, an early indicator of atherosclerosis, compared with control rabbits.<sup>34</sup>

Gordon et al<sup>35</sup> performed yet another study investigating atherosclerosis in rabbits fed a high-cholesterol diet. Four groups of rabbits were used: two groups were subjected to endothelial denudation by a balloon catheter and were fed a high-cholesterol diet, and 2 groups were used as controls. DHEA was administered to 1 injured group and to 1 control group. Rabbits given a high-cholesterol diet and DHEA showed a 48% reduction in atherosclerotic plaque size compared with rabbits given a high-cholesterol diet and no DHEA. Reduction of plaque size by DHEA administration was not seen in the groups fed standard chow. There were no significant differences found in food intake, weight, and cholesterol levels in each group, further suggesting that DHEA may inhibit the development of atherosclerosis.<sup>35</sup>

Nathan et al<sup>36</sup> suggested that aromatase, an enzyme that converts testosterone to estrogens, may play an important role in limiting atherosclerosis in males. In this study, male

mice were orchietomized or underwent a sham operation. Orchietomized mice were given placebo, testosterone, testosterone plus aromatase inhibitor, or estrogen. Sham-operated mice were given aromatase inhibitor or nothing. There were significantly fewer lesions in the testes-intact group and the orchietomy plus testosterone group than in the testes-intact plus aromatase inhibitor group, the orchietomized plus placebo group, and the orchietomized plus testosterone plus aromatase inhibitor group, suggesting that testosterone and aromatase are both necessary for attenuating atherosclerosis. In this study, an increase in testosterone also decreased LDL with no change in high-density lipoprotein concentration.<sup>36</sup> This may lead to a potentially new therapeutic methodology for the treatment of atherosclerosis and dyslipidemia: testosterone plus aromatase administration.

Hatakeyama et al<sup>37</sup> described the effect of testosterone on tumor necrosis factor alpha (TNF $\alpha$ )-induced expression of vascular cell adhesion molecule 1 (VCAM-1) in human aortic endothelial cells (HAECs). Because VCAM-1 plays an important role in the recruitment of white blood cells into arterial walls, the inhibition of such would attenuate atherogenesis and therefore inhibit atherosclerosis. This study showed that incubation of HAECs with testosterone yielded a dose-dependent reduction in VCAM-1 expression that was negated with the introduction of an androgen receptor blocker. Because VCAM-1 expression decreased, the recruitment of white blood cells into the arterial wall was diminished, and thus atherogenesis was inhibited.<sup>37</sup>

In contrast to the studies described above, some studies show that exogenous testosterone exacerbates atherosclerosis. McCrohon et al<sup>38</sup> examined human white blood cells and umbilical vein endothelial cells exposed to DHT (40 or 400 nmol/L), flutamide or control. Blood cells and endothelial cells exposed to DHT were treated with or without flutamide. In this study, white blood cells were more likely to adhere to endothelial cells in the presence of testosterone compared with control. This dose-dependent effect was blocked by flutamide. This study also showed that DHT increased VCAM-1 expression, which is contrary to previously described studies. Researchers found similar results when these methods were tested on arterial endothelium, suggesting that DHT increases atherogenesis.<sup>38</sup> This study differs from the previous studies in one obvious way: only endothelial cells from veins were used in this study as opposed to the entire artery in other studies. This suggests that there is an important mechanism that occurs within the tunica media, a layer not found in veins, of the artery that attenuates atherogenesis and atherosclerosis.

Ng et al<sup>39</sup> examined the effect of DHT on human macrophages from both male and female donors in a similar study. Researchers investigated atherosclerosis-related gene expres-

sion by real-time reverse transcription polymerase chain reaction (RT-PCR) in the presence of DHT. Results of this study showed that treatment with DHT caused upregulation of 27 proatherosclerotic genes in macrophages from male donors, with negative functional consequences. In contrast, no proatherosclerotic genes were upregulated in macrophages from female donors. This study suggests that DHT, a nonaromatizable form of testosterone that has a high affinity for the androgen receptor, only alters macrophage genes in males.<sup>39</sup> This may be an important part of the testosterone–atherosclerosis relationship, which was overlooked in this study, as the aromatization of testosterone to estrogens has been shown to inhibit atherosclerosis in male mice.<sup>36</sup>

The studies describing the relationship between testosterone and atherosclerosis have brought about controversy. Many studies suggest that testosterone inhibits atherosclerosis,<sup>31–37</sup> whereas some studies suggest that testosterone may be detrimental.<sup>38,39</sup> Because of this discrepancy, more research is necessary to completely understand the association between testosterone and atherosclerosis. Much of this information can be seen in Table 2.

## Vasoconstriction

Even though there is much evidence suggesting that testosterone may cause arterial vasodilation,<sup>17–22</sup> some studies suggest that testosterone may constrict blood vessels under certain circumstances. After noticing a correlation between anabolic steroid use and increased adverse cardiovascular events such as sudden myocardial infarction in athletes, Ammar et al<sup>40</sup> set out to investigate and determine the validity of this phenomenon in a rabbit model. Male rabbits were given a weekly intramuscular injection of testosterone (25 mg/kg) or no treatment. The descending thoracic aorta was excised and either contracted with 5-hydroxytryptamine (5-HT) or dilated with sodium nitroprusside (SNP). When treated with 5-HT, aortas from the testosterone group displayed statistically significantly higher contraction percentages than those from the control group. When treated with SNP, the testosterone group had a significantly lower relaxation percentage than did the controls at testosterone concentrations  $\geq 10^{-6}$  mol/L.<sup>40</sup> These findings suggest that testosterone attenuates dilation and intensifies constriction of the blood vessels.

Ceballos et al<sup>41</sup> conceded that testosterone can induce relaxation of the aorta and coronary arteries, but they also believed that testosterone may facilitate vasoconstriction. Isolated and perfused rat hearts (Langendorff) were examined to expose the effects of testosterone in the coronary vasculature. This study showed that testosterone partially inhibits the vasodilatory effect of adenosine by increased vascular resistance, which leads to decreased flow. Vessels

**Table 2.** Effects of Testosterone on Atherosclerosis

Study	Effect	Model	End Points Measured	Major Findings
Hanke et al <sup>32</sup>	Positive	Rabbit	<ul style="list-style-type: none"> <li>Neointimal plaque levels in the presence/absence of testosterone and endothelium.</li> <li>Androgen receptor expression.</li> </ul>	<ul style="list-style-type: none"> <li>Testosterone inhibited neointimal plaque regardless of endothelial denudation.</li> <li>Testosterone induced a 50% increase in androgen receptor mRNA expression.</li> </ul>
Alexandersen et al <sup>33</sup>	Positive	HCD rabbit	<ul style="list-style-type: none"> <li>Atherosclerotic plaque development.</li> </ul>	<ul style="list-style-type: none"> <li>Atherosclerotic plaque was inhibited by testosterone administration compared with 0 control.</li> </ul>
Arad et al <sup>34</sup>	Positive	HCD rabbit	<ul style="list-style-type: none"> <li>Atherosclerotic plaque development.</li> </ul>	<ul style="list-style-type: none"> <li>DHEA administration decreased atherosclerotic plaque by 40% compared with control.</li> </ul>
Gordon et al <sup>35</sup>	Positive	HCD rabbit	<ul style="list-style-type: none"> <li>Atherosclerotic plaque development.</li> </ul>	<ul style="list-style-type: none"> <li>DHEA administration decreased atherosclerotic plaque by 48% compared with control.</li> </ul>
Nathan et al <sup>36</sup>	Positive	Mouse	<ul style="list-style-type: none"> <li>Number of atherosclerotic lesions.</li> </ul>	<ul style="list-style-type: none"> <li>Lesion count was lower in normal, O+T, and O+E animals compared with other groups.</li> <li>Aromatase inhibitor voided the beneficial effect of testosterone.</li> </ul>
Hatakeyama et al <sup>37</sup>	Positive	HAECs	<ul style="list-style-type: none"> <li>VCAM-1 expression in the presence/absence of testosterone.</li> </ul>	<ul style="list-style-type: none"> <li>HAEC incubation in testosterone decreased VCAM-1 expression.</li> <li>Decreased VCAM-1 would inhibit atherogenesis.</li> </ul>
McCrohon et al <sup>38</sup>	Negative	HWBCs and HUVECs	<ul style="list-style-type: none"> <li>HWBC endothelial adherence and VCAM-1 expression in the presence/absence of DHT, HF.</li> </ul>	<ul style="list-style-type: none"> <li>DHT increased WBC adherence to the endothelium.</li> <li>DHT increased VCAM-1 expression.</li> </ul>
Ng et al <sup>39</sup>	Negative	Human macrophages	<ul style="list-style-type: none"> <li>Atherosclerotic gene expression in the presence/absence of DHT.</li> </ul>	<ul style="list-style-type: none"> <li>27 Proatherosclerotic genes expressed in the presence of DHT in macrophages from male donors.</li> </ul>

HCD indicates high-cholesterol diet; O, orchietomized; DHEA, dehydroepiandrosterone (testosterone analogue); O+T, orchietomized plus testosterone; O+E, orchietomized plus estrogen; VCAM, vascular cell adhesion molecule; HAECs, human aortic endothelial cells; HWBCs, human white blood cells; HUVECs, human umbilical vein endothelial cells; HF, hydroxyflutamide; DHT, dihydrotestosterone.

dilated with adenosine showed significantly more dilation than with adenosine plus testosterone. These authors also acknowledged the previously described vasodilatory effects of testosterone. They suggested that the discrepancies in results are a result of differences in the way testosterone interacts with different parts of the vessel. They further proposed that testosterone may compete with other vasodilatory compounds in a way that induces vasoconstriction.<sup>41</sup> Ceballos et al did not investigate a mechanism in this study.

## Inflammation

During early remodeling of the left ventricular free wall after myocardial injury, inflammation may cause a fatal rupture, and males have a higher rupture rate than females.<sup>42</sup> In an effort to define the differences between the sexes in cardiac rupture rate, Cavasin et al<sup>43</sup> investigated neutrophil infiltration in the myocardial infarct border zone in mice to measure potential sex differences in inflammation. The mice were divided by sex and gonadectomy or sham gonadectomy. All males were administered either estrogen or placebo, whereas all females were administered either testosterone or placebo. Testosterone was

given at 208  $\mu\text{g}/\text{day}$  for 60 days to achieve approximately normal male physiological levels. Mice were then subjected to an LAD ligation. Both intact and orchietomized female mice receiving testosterone had significantly higher rates of neutrophil infiltration in the myocardial infarct border zone on day 1 after myocardial infarction compared with placebo. This difference was mitigated after day 1. In males, the testes-intact plus placebo group had a significantly higher neutrophil density than the testes-intact male plus estrogen group for the first 2 days after MI, suggesting estrogen inhibited neutrophil infiltration. These data suggest that testosterone alone increases neutrophil infiltration and therefore may increase inflammation when compared with mice without testosterone.<sup>43</sup> Increased inflammation following acute myocardial infarction may lead to increased rupture in the myocardium. Cavasin et al also showed that females in each testosterone group (ovariectomy and sham-ovariectomy) had a significantly higher mortality rate secondary to cardiac rupture compared with the placebo groups. In males, the only significant difference was between the sham-castration plus placebo group and the castration plus placebo group. The sham-castration group had a higher rupture and death rate, which also suggests that testosterone

exacerbates the inflammatory response and the potential for cardiac rupture after MI.<sup>43</sup>

Crisostomo and coworkers<sup>44</sup> arrived at similar conclusions. When compared with controls, female and castrated male rats given a testosterone infusion before ischemia had an increased expression of active p38 and SPAK/JNK, which are signaling proteins associated with myocardial inflammation.<sup>44</sup> This supports the notion that testosterone enhances inflammation.

The role of testosterone in inducing inflammation may not be limited to increased p38 and SPAK/JNK signaling protein expression. Rettew et al<sup>45</sup> investigated toll-like receptor-4 (TLR-4) expression in mice, as TLR-4 has been shown to mediate various immune responses.<sup>46</sup> In this study, macrophages were isolated from orchiectomized, sham-orchiectomized, and orchiectomized plus testosterone-replaced mice. This study showed a small but statistically significant decrease in TLR-4-mediated inflammatory cytokine production by isolated macrophages in the orchiectomized plus testosterone group compared with the orchiectomized group. Contrary to other reports, this study suggested that testosterone decreases the inflammatory response in mice, which may favorably affect early cardiac remodeling after MI.<sup>45,47</sup>

Not all studies involving inflammatory pathways are cardiovascular in nature, as seen in Table 3. To better understand the increased incidence of autoimmune diseases in females compared with males, Bebo et al<sup>48</sup> examined the inflammatory factors in autoimmune encephalomyelitis in mice. The nervous and cardiovascular systems share common inflammatory pathways. Researchers in this study found that testosterone significantly decreased the interferon- $\gamma$ /interleukin-10 (IFN- $\gamma$ /IL-10) ratio by decreasing IFN- $\gamma$  and Th1 proinflammatory cytokines and increasing IL-10 concentration.<sup>48</sup> This suggests that testosterone attenuates the IFN- $\gamma$  inflammatory pathway and enhances the IL-10 pathway.

In a similar study, Liva and Voskuhl<sup>49</sup> examined male and female mice treated with DHT or placebo. Isolated rat splenic cells were examined in this study. Testosterone-treated splenic cells showed increased IL-10 secretion compared with cells treated with placebo. As IL-10 is a Th2 anti-inflammatory cytokine, this study suggested that testosterone triggered a reduction in inflammation. T lymphocytes, B lymphocytes, and macrophages can produce IL-10; thus, more research was necessary to determine the source of the IL-10 increase. The results of this second investigation showed that additional IL-10 is synthesized by CD4<sup>+</sup> lymphocytes. The authors suggested that testosterone may act directly on CD4<sup>+</sup> lymphocytes.<sup>48</sup> This may lead to advancements in MI therapy, as inflammation is one of the factors of early remodeling, which causes the left ventricle to dilate and become less efficient.<sup>47</sup> Attenuating myocardial remodeling may lead to improved ejection fraction and long-term survival in patients with MI.<sup>47</sup>

An important mediator of bone loss, interleukin-6 (IL-6) also has proinflammatory properties. Because estrogen deficiency is a principal cause of bone loss, Bellido et al<sup>50</sup> examined the effects of testosterone on IL-6 as it pertains to osteoblastic cell lines. What this study showed was that an increase in either testosterone or DHT inhibited IL-6 production dependent on the androgen receptor, which then led to a decrease in inflammation.<sup>50</sup> Hofbauer et al<sup>51</sup> also showed testosterone and DHT at a 10<sup>-7</sup> mol/L concentration inhibit IL-6 mRNA expression. Because equimolar doses of estrogen had no effect on IL-6 mRNA expression, the researchers suggested that the androgen receptor may facilitate the effects of testosterone during the conversion of testosterone to DHT, a nonaromatizable androgen.<sup>50</sup>

Flake et al<sup>52</sup> determined the effects of sex hormones on inflammation of the temporomandibular joint in male and female rats. Male and female rats were either left intact,

**Table 3.** Effects of Testosterone on Inflammation in Noncardiovascular Basic Science Models

Study	Model	End Points Measured	Major Findings
Bebo et al <sup>48</sup>	Mouse experimental autoimmune encephalomyelitis	<ul style="list-style-type: none"> <li>IFN-<math>\gamma</math> expression</li> <li>IL-10 expression</li> <li>IFN-<math>\gamma</math>/IL-10 ratio</li> </ul>	<ul style="list-style-type: none"> <li>IFN-<math>\gamma</math> expression is decreased in the presence of testosterone.</li> <li>IL-10 expression is increased in the presence of testosterone.</li> <li>Inflammation is inhibited in the presence of testosterone.</li> </ul>
Liva and Voskuhl <sup>49</sup>	Rat splenic cells	<ul style="list-style-type: none"> <li>IL-10 expression</li> <li>Mechanism of action</li> </ul>	<ul style="list-style-type: none"> <li>IL-10 expression increased in the presence of testosterone.</li> <li>IL-10 production comes from CD4<sup>+</sup> T lymphocytes.</li> </ul>
Bellido et al <sup>50</sup>	Osteoblastic cell lines	<ul style="list-style-type: none"> <li>IL-6 expression</li> </ul>	<ul style="list-style-type: none"> <li>IL-6 expression was decreased in the presence of testosterone; inflammation was decreased.</li> </ul>
Hofbauer et al <sup>51</sup>	Osteoblastic cell lines	<ul style="list-style-type: none"> <li>IL-6 expression</li> </ul>	<ul style="list-style-type: none"> <li>IL-6 expression was decreased in the presence of testosterone; inflammation was decreased.</li> </ul>
Flake et al <sup>52</sup>	Rat TMJ	<ul style="list-style-type: none"> <li>Plasma extravasation to assess the inflammatory response</li> </ul>	<ul style="list-style-type: none"> <li>Testosterone increased plasma extravasation, showing an increased inflammatory response.</li> </ul>

IFN indicates interferon; IL, interleukin; TMJ, temporomandibular joint.



underwent a gonadectomy, or underwent a gonadectomy plus exogenous hormone treatment. In intact males, testosterone exacerbated inflammation compared with in orchiectomized males, further increasing the controversy of testosterone and its role in modulating the inflammatory response.<sup>51</sup>

## Death Signaling

Heart disease is the leading cause of death in both sexes.<sup>53</sup> The concept of cardiomyocyte death signaling during ischemic heart disease and the role of testosterone merits investigation. Crisostomo et al<sup>44</sup> performed a study examining testosterone and death signaling in rats. Four groups of rats were studied: normal females, females subjected to an acute testosterone infusion, orchiectomized males, and orchiectomized males subjected to an acute testosterone infusion. Testosterone infusions, 5 minutes prior to myocardial ischemia, were given at a concentration of 10 ng/mL, approximately physiological levels. The hearts were isolated, then subjected to 25 minutes of ischemia followed by 40 minutes of reperfusion. Western blot analysis revealed upregulation of caspase-3 (apoptotic) and downregulation of Bcl-2 (antiapoptotic) in the testosterone group compared with the controls.<sup>44</sup> Estrada et al and Jia et al found similar effects of testosterone in the caspase-3 and Bcl-2 pathways, respectively, further suggesting a proapoptotic function of testosterone.<sup>54,55</sup>

To further investigate the effects of testosterone on apoptosis, Wang et al<sup>56</sup> isolated hearts from adult male rats, orchiectomized male rats, and testes-intact male rats given flutamide. Isolated hearts were subjected to 25 minutes of myocardial ischemia followed by 40 minutes of reperfusion. Heart tissue was then analyzed for apoptotic and inflammatory signaling proteins. Researchers found that after ischemia and reperfusion, castrated male and flutamide-treated male hearts showed decreased caspase-1, caspase-3, caspase-11, TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and activated p38 MAPK in conjunction with increased Bcl-2 expression. When compared with the control (intact) males, both groups of treated males showed decreases in proapoptotic signaling, suggesting that testosterone is proapoptotic and is harmful to cardiomyocytes during ischemia and reperfusion.<sup>56</sup>

Huang et al<sup>57</sup> came to similar conclusions. Rat hearts from males, females, castrated males, males given flutamide, castrated males given chronic DHT implantation, and castrated males given acute testosterone infusion (ATI) were subjected to 25 minutes of ischemia and 40 minutes of reperfusion in vitro. Compared with normal males, castration and flutamide treatment improved cardiac function after ischemia via upregulation of the protein kinase B (Akt) pathway (a prosurvival, antiapoptosis pathway) in animals with no endogenous testosterone and blocked androgen receptors. Increases in p-Akt, p-Bad, and Bcl-2 expression in castrated males and flutamide-treated

males compared with intact males suggest greater prosurvival pathway activation in testosterone-deficient rat hearts.<sup>57</sup> These findings suggest that testosterone attenuates the Akt pathway, which may lead to increased apoptosis and necrosis and reduced cardiac function after myocardial infarction.

Existing evidence strongly suggests a proapoptotic effect of testosterone. Studies show both a decrease in the Akt prosurvival pathway in testosterone-treated male animals and upregulation of the Akt prosurvival pathway in male animals without endogenous testosterone or with a blocked androgen receptor, suggesting that testosterone gives way to an increase in death signaling and therefore attenuates cardiomyocyte survival.

## Conclusion

Because of the increase in the number of prescriptions and use of testosterone in adult males for the treatment of hypogonadism, low libido, and weakness,<sup>1</sup> an investigation of the effects of testosterone on the cardiovascular system in basic science studies was carried out. The benefits of testosterone relating to the cardiovascular system are as follows. Testosterone has been shown to exhibit potential antiarrhythmic properties in the form of decreasing action potential duration, early after depolarizations, and shortened QTc interval.<sup>5-7,9</sup> Testosterone has also been shown to reduce myocardial infarct size compared with that in subjects not treated with testosterone by modulating the myocardial K<sub>ATP</sub> channel,<sup>8,10,11,16</sup> enhancing vasodilation,<sup>17-23</sup> improving lipid metabolism,<sup>24,25</sup> and improving DM.<sup>27,28</sup> These conclusions do not come without controversy. Much of the literature suggests that testosterone attenuates atherosclerosis,<sup>31-37</sup> but some studies suggest otherwise.<sup>38,39</sup> Further deleterious effects of testosterone on the cardiovascular system have been shown in studies on vasoconstriction,<sup>40,41</sup> inflammation,<sup>43-45,48-52</sup> and death signaling.<sup>43,51-55</sup> These findings suggest that testosterone may simultaneously benefit and harm the cardiovascular system by different pathways. The complexity of this relationship is obvious, and thus additional basic science studies are required for a better understanding of the relationship between testosterone and the cardiovascular system.

## Disclosures

None.

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**Key Words:** arrhythmia • atherosclerosis • infarction  
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