The American Journal of Pathology, Vol. ■, No. ■, ■ 2023

The American Journal of PATHOLOGY
ajp.amjpathol.org

REVIEW

Understanding One Half of the Sex Difference Equation

The Modulatory Effects of Testosterone on Diabetic Cardiomyopathy

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Accepted for publication November 20, 2023.

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Diabetes is a prevalent disease, primarily characterized by high blood sugar (hyperglycemia). Significantly higher rates of myocardial dysfunction have been noted in individuals with diabetes, even in those without coronary artery disease or high blood pressure (hypertension). Numerous molecular mechanisms have been identified through which diabetes contributes to the pathology of diabetic cardiomyopathy, which presents as cardiac hypertrophy and fibrosis. At the cellular level, oxidative stress and inflammation in cardiomyocytes are triggered by hyperglycemia. Although males are generally more likely to develop cardiovascular disease than females, diabetic males are less likely to develop diabetic cardiomyopathy than are diabetic females. One reason for these differences may be the higher levels of serum testosterone in males compared with females. Although testosterone appears to protect against cardiomyocyte oxidative stress and exacerbate hypertrophy, its role in inflammation and fibrosis is much less clear. Additional preclinical and clinical studies will be required to delineate testosterone's effect on the diabetic heart. *(Am J Pathol 2023, ■: 1–11; https://doi.org/10.1016/j.ajpath.2023.11.009)*

Diabetes is a prevalent disease, affecting 8.8% of the world's population.[1] Patients with diabetes have an increased risk of cardiovascular diseases, including atherosclerotic cardiovascular disease and heart failure.[2,3] The greater incidence of myocardial dysfunction in diabetic patients, even in those free from coronary artery disease or hypertension, indicates that diabetes negatively affects the heart independent of these pathologies.[4–7] Diabetic cardiomyopathy (DbCM) is defined as a disease of the myocardium independent from hypertension or coronary artery disease.[4,7,8]

DbCM is characterized by left ventricular hypertrophy, diastolic dysfunction, and myocardial fibrosis, which may progress to heart failure.[4,7,9,10] A study of patients from the UK Biobank Cardiovascular Magnetic Resonance Substudy found significant remodeling in all four chambers of the heart. Reduced LV and RV volumes were noted, even at an early stage of disease.[11] Similarly, myocardial capillary basement membrane thickening and accumulation of lipid droplets were apparent in hearts of diabetic patients.[12] Many of these effects are thought to be caused by the impact of hyperglycemia and hyperlipidemia on cardiomyocytes.[4,13] The cellular mechanisms underlying DbCM are multifactorial and include oxidative stress, fibrosis, hypertrophy, and inflammation.[4,13]

Testosterone is a steroid hormone and the major sex hormone in males.[14,15] It is produced in Leydig cells in the

Supported by Canadian Institutes of Health Research operational grant PJT-166092. G.H.W. holds the ISTH-McMaster Chair in Thrombosis and Hemostasis Research, and is supported by an HSFC Ontario Mid-Career Investigator Award.

https://doi.org/10.1016/j.ajpath.2023.11.009

testicles, and synthesis is stimulated through the hypothalamic-pituitary-gonadal axis.[14,15] The normal serum testosterone level for males ranges from 1.86 to 11.18 μg/L.[16,17] Circulating testosterone diffuses through the cell membrane and binds the androgen receptor (AR) in the cytoplasm of most cells (Figure 1B).[14] The testosterone-AR complex then translocates into the nucleus and binds androgen-response elements within the promoter regions of target genes, leading to the recruitment of coregulatory proteins and the enhanced transcription of these target genes.[14] Impacts of testosterone include male sexual development, erythropoiesis, muscle growth, and increased bone density.[15]

Although it has been established that estrogens play a protective role in heart function, the effect(s) of testosterone is less well-defined.[18] A recent trial found that testosterone replacement therapy in men with hypogonadism and cardiovascular risk factors did not increase the incidence of major adverse cardiac events.[19] However, because all patients in this study were over age 45 years, there is yet to be an adequately powered randomized control trial evaluating the use of this therapy in younger men.[20] Additionally, males are more likely to develop cardiovascular disease than females and levels of testosterone are positively correlated with the development of heart failure in males.[21,22]

There exist notable sex differences in the development and progression of DbCM.[23] Interestingly, although males are more likely to develop diabetes than females,[23] data pooled from 64 cohorts, including over 800,000 individuals, found that diabetic females have a greater risk of developing coronary heart disease than diabetic males.[24] In addition, the risk of cardiomyopathy is higher in diabetic females than in diabetic males.[23] Unfortunately, limited evidence exists from human subjects to define the impact of sex hormones on cellular processes responsible for DbCM (Figure 1B).[23] Therefore, further research into the impacts of diabetes and testosterone on cardiomyocytes is required to direct the development of more effective therapeutic strategies.

The objective of this review is to identify potential interactions between the actions of testosterone and hyperglycemia in cardiomyocytes (Figure 1A). This review focuses on four mechanisms associated with DbCM: hypertrophy, oxidative stress, inflammation, and fibrosis. Particular attention will be placed on studies attempting to delineate the influence of hyperglycemia/hyperlipidemia on cardiomyocytes.

Oxidative Stress

It is well-established that diabetes is associated with the occurrence of oxidative stress throughout the body, including the heart.[13,25] Oxidative stress is hypothesized to contribute to cardiomyocyte apoptosis and the development of heart failure.[10,18] Oxidative stress is caused by reactive oxygen species (ROS), including free radicals, hydrogen peroxide, and superoxide, which can react with, and damage DNA, proteins, and lipids in cardiomyocytes.[26] *In vitro*, elevated concentrations of glucose are associated with the development of ROS and apoptosis of cardiomyocytes in a concentration- and time-dependent manner.[27,28] Similarly, STZ-injected animals have higher levels of ROS in the heart.[29,30]

Testosterone has been shown to exert both pro- and antioxidant effects.[31] In the presence of hyperglycemia, the removal of testosterone by castration increased STZ-associated oxidative stress in rats, whereas supplementation with exogenous testosterone reduced ROS levels.[32] This result suggests a potential antioxidant/protective role for testosterone in the context of diabetes.

Glutathione (GSH) scavenges ROS and is converted to glutathione disulfide (GGSG), in a reaction catalyzed by glutathione-peroxidase (GSH-Px).[31,33] STZ-injected rats were shown to have a reduced ratio of GSH:GGSG, as well as higher levels of GSSG compared with controls, suggesting increased oxidative stress.[30] Although one study found diminished GSH-Px activity levels in the hearts of diabetic mice models, another found no difference in GSH-Px activity between STZ-injected rats and controls.[24,28] In STZ-injected rats, castration was shown to lower protein levels of GSH-Px in the heart, which was restored by testosterone supplementation.[32] GSH-Px activity was decreased by testosterone supplementation in nondiabetic animals, concomitant to increased oxidative stress.[34–36] Testosterone was able to restore GSH-Px activity in one study, but not another.[34,35]

Catalase is an antioxidant enzyme used to detoxify hydrogen peroxide by converting it into water and hydrogen.[37] STZ-injected rats were found to have decreased catalase protein levels and activity, compared with control rats, as well as signs of oxidative stress.[30] Testosterone supplementation was able to increase catalase protein levels in the hearts of diabetic rats.[32] By contrast, other work in nondiabetic animals have shown that testosterone propionate injection decreased the activity of catalase.[35] Another study found that gonadectomy decreased catalase activity, though testosterone propionate supplementation caused a further decrease.[34]

Mice fed a high-fat diet and injected with STZ were observed to have decreased levels of superoxide dismutase (SOD), which scavenges superoxide.[26,29] Decreased SOD activity was also observed in nondiabetic mice injected with testosterone propionate; however, testosterone supplementation in the diabetic model increased SOD protein levels in the heart.[32,35] The reasons for the apparent differential effects of testosterone on oxidative stress in diabetic and healthy animals should be investigated.

Nuclear factor-erythroid factor 2-related factor 2 (Nrf2) is a transcription factor and part of a cellular defense system against oxidative stress.[38,39] Nrf2 increases translation of genes that code for antioxidant enzymes, such as *GSH-PX*, *GSH*, *catalase*, and *SOD*.[40] Cardiomyocytes cultured in

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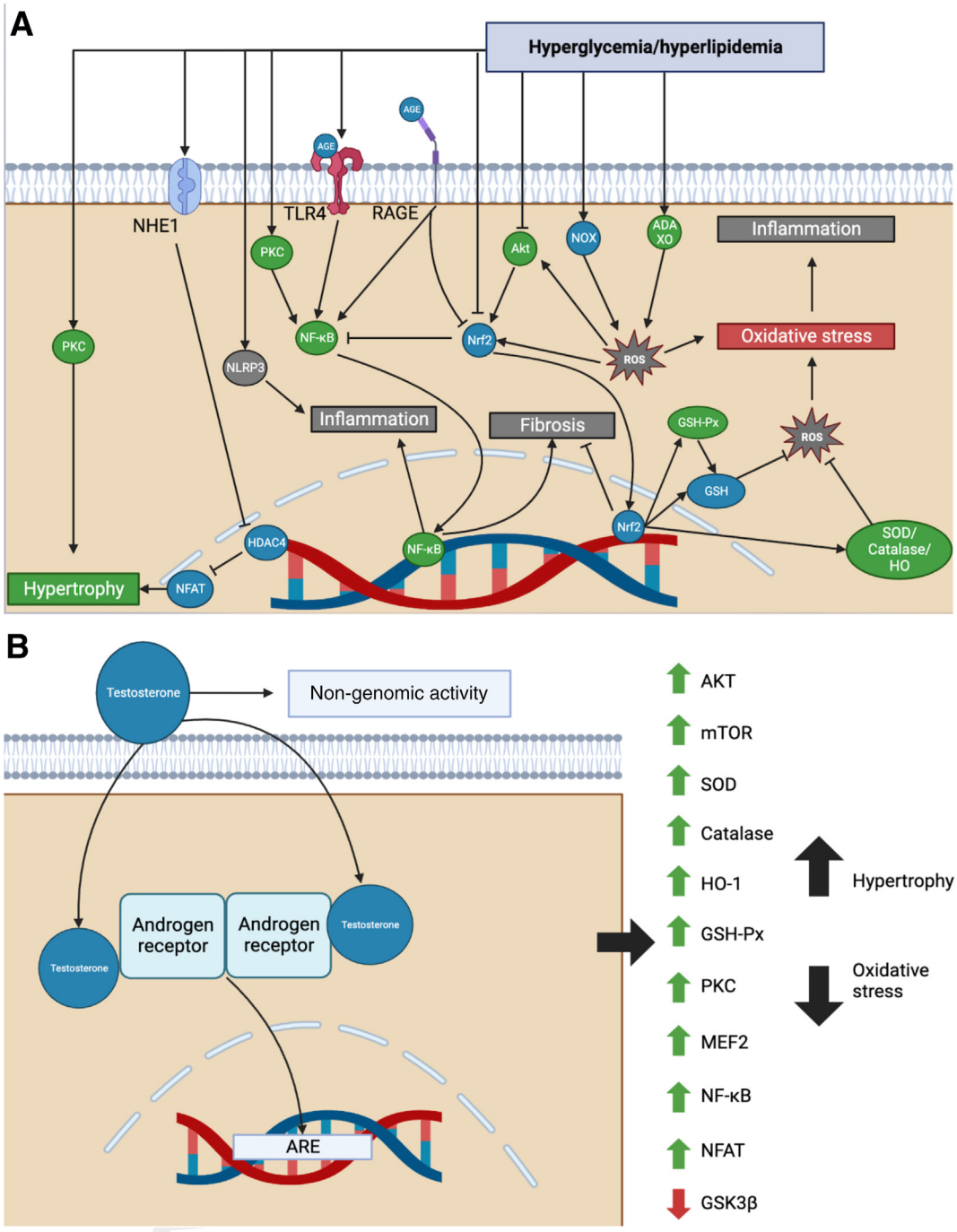

Figure 1 **A:** Testosterone signaling. Testosterone binds to the androgen receptor in the cytoplasm, forming the androgen receptor (AR) complex. Upon dimerization, this complex translocases to the nucleus, binding to androgen response elements (AREs) in the promoters of specific gene thereby regulating gene transcription. Testosterone impacts numerous metabolic pathways generally promoting hypertrophy and attenuating oxidative stress in cardiomyocytes. **B:** Pathways involved in the pathogenesis of diabetic cardiomyopathy (DbCM), with the impacts of testosterone highlighted. Pathways positively regulated by testosterone are indicated in green, whereas negative regulation is indicated in red. ADA, adenosine deaminase; AGE, advanced glycation end products; GSH-Px, glutathione peroxidase; GSK-3β, glycogen synthase kinase-3β; HDAC4, histone deacetylase-4; HO-1, heme oxygenase-1; KC, protein kinase C; MEF2, myocyte enhancer factor-2; mTOR, mammalian target of rapamycin; NFAT, nuclear factor of activated T-cells; NHE-1, sodium-hydrogen exchanger-1; NLRP3, nucleotide-binding oligomerization domain like receptor pyrin domain containing 3; NOX, NADPH oxidase; Nrf2, nuclear factor-erythroid factor 2-related factor 2; PKC, protein kinase C; RAGE, receptor for AGEs; ROS, reactive oxygen species; SOD, superoxide dismutase; TLR4, toll-like receptor 4; XO, xanthine oxidase. Figure created with Biorender.com (Toronto, ON, Canada).

high glucose and palmitate had increased *Nrf2* expression.[41] In STZ-injected mice, a transient increase in *Nrf2* expression and greater nuclear translocation of the protein in the presence of hyperglycemia was noted, though over time, reduced levels were observed compared with controls.[42,43] This suggests a protective increase in the early stages of

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the disease, which is eventually attenuated by the progression of the pathology.[43]

Heme oxygenase-1 (*HO-1*) is another gene regulated by Nrf2. HO-1 is responsible for degrading heme into biliverdin, carbon monoxide, and free iron, a process that ultimately reduces ROS.[44,45] In the hearts of STZ-injected animals, increased expression of *HO-1* and *HO-2* were noted.[46,47] Surprisingly, the inhibition of HO activity by chemical inhibitor tin protoporphyrin IX (SnPPIX) diminished oxidative stress.[46] However, the method of HO inhibition may have effects on systems other than HO, which are responsible for the reduction in stress. Conversely, another study found that systemic overexpression of *HO-1* reduced oxidative stress in the heart.[47] Therefore, it is most likely that HO is protective against oxidative stress in the context of diabetes.[46,47] Testosterone deficiency was associated with a reduction in HO-1 protein levels and HO activity, and increased oxidative stress, leading to cardiac damage. Testosterone supplementation was found to increase the protein levels of HO-1, increase HO activity, and decrease levels of oxidative stress.[48]

Akt expression in cardiomyocytes is known to be increased in the presence of oxidative stress, and Akt positively regulates Nrf2 activity.[49,50] This increase is enhanced by testosterone, so increased Akt activity may partially explain some of testosterone's protective effects.[50] However, in the context of diabetes, reduced Akt phosphorylation is well-described, coinciding with decreased activity.[41,47] Therefore, potential protective effects of testosterone may be blunted in diabetic models. This underscores the need for experiments delineating the impact of testosterone on mechanisms associated with oxidative stress in diabetic models.

Adenosine deaminase (ADA) and xanthine oxidase (XO) are involved in a pathway that generates uric acid, producing ROS as a byproduct.[51] Hearts of STZ-injected rats have increased XO and AO activity compared with healthy controls.[52] XO and ADA activity have an impact on oxidative stress in the context of diabetic animal models. Blocking XO activity or the formation of its substrate has been shown to reduce free radical generation and oxidative stress in the heart, including in the animal models of diabetes.[51,53,54] Similarly, administration of testosterone to late-gestational rats led to increase oxidative stress in conjunction with increased ADA/XO activity.[55] This suggests that testosterone can synergistically exacerbate oxidative stress through increasing XO activity. Whether testosterone has similar effects on oxidative stress through this pathway in diabetic models should be investigated.

NF-κB is a transcription factor associated with inflammation and is activated by hyperglycemia and hyperlipidemia associated with diabetes.[56] NF-κB signaling can be activated by glucose-associated oxidative stress by signaling through toll-like receptors (TLRs), specifically TLR4, which is the most abundant TLR in the myocardium.[57–59] Cardiomyocytes cultured in high glucose had increased TLR4 and NF-κB protein, and addition of a ROS scavenger led to decreased TLR-4 protein expression.[59,60] TLR4 mRNA and protein levels were increased in STZ-injected animals, in conjunction with oxidative stress.[60,61] Gene silencing of *TLR4* in STZ-treated mice led to reduced ROS production.[61] Evidence suggests that Nrf2 can inhibit NF-κB signaling.[38] HO-1 is believed to be a key mediator, and *HO-1* overexpression was able to reduce NF-κB DNA binding activity in cardiomyocytes.[38,62]

NF-κB signaling in cardiomyocytes was found to be activated by testosterone through an AR-dependent mechanism, which protected against superoxide-induced apoptosis.[50] Because oxidative stress was elicited by superoxide in this study, not high glucose, testosterone's impact in diabetes may vary. It is plausible that NF-κB has opposing effects on oxidative stress, depending on the cell conditions. For instance, anti-oxidants that are targets of NF-κB signaling include *HO-1*, *GSH-Px*, and *SOD*.[63] Conversely, NF-κB is also known to increase the expression of NADPH oxidase (NOX) subunits and XO.[38,63] Specifically, the protective effect of testosterone may be due to increased Akt activity, which is known to have antioxidant functions.[49] The increase in Akt upon testosterone administration was blunted by inhibiting NF-κB signaling.[50] However, in the context of diabetes, inhibition of Akt is well-recognized.[58] Therefore, NF-κB signaling may be harmful in cardiomyocytes exposed to high glucose because the inhibition of Akt blunts a protective role, whereas its pro-oxidative effects remain intact.

There are some pathways through which oxidative stress is stimulated by DbCM on which the impact of testosterone has not been investigated. NOX is responsible for the generation of ROS through the production of superoxide and hydrogen peroxide.[58,64] Mouse models of diabetes have Q9 increased NOX expression and NOX-activated NF-κB in cardiomyocytes cultured in high glucose.[65–67] Advanced glycation end products (AGEs) are proteins or lipids that are glycated after exposure to elevated concentrations of glucose or as a result of oxidative stress.[6,68,69] Knockout of the receptor for AGEs (*RAGE*) in Western diet–fed mice reduced superoxide production, suggesting that AGE accumulation also leads to oxidative stress.[70] Similarly, cardiomyocyte exposure to AGE led to increased NF-κB nuclear translocation, decreased *Nrf2* expression and nuclear translocation of the protein, and decreased *HO-1* expression.[71]

Inflammation

Inflammation represents the body's response to injury or infection.[72] Inflammatory pathways are activated when pattern recognition receptors bind damage-associated molecular patterns or pathogen-associated molecular patterns.[72] This leads to the release of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), and IL-1β, which

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activate immune cells.[72] These cytokines also stimulate the expression of intracellular adhesion molecule 1 (*ICAM-1*) and vascular cell adhesion molecule 1 (*VCAM-1*) on endothelial cells, which allow for leukocyte migration into the tissue, enhancing the inflammatory response.[72]

In the context of myocardial damage, cardiomyocytes are one of the first cells to show inflammatory activity, with the release of proinflammatory cytokines leading to recruitment of circulating immune cells.[57] Inflammation in diabetes is stimulated by hyperglycemia and high concentrations of free fatty acid in the blood.[73] Evidence suggests that oxidative stress may mediate the progression from hyperglycemia to inflammation.[74,75] Inflammatory activity in the myocardium can lead to fibrosis, which leads to myocardial stiffness and diastolic dysfunction.[76]

The presence of sex differences in the occurrence inflammatory disease is well-established, with women generally at a greater risk than men.[77] Some clinical trials of testosterone replacement therapy have identified an inverse relationship between testosterone levels and proinflammatory cytokines, whereas others have found no relationship.[78] Focusing on the direct inflammatory influences of testosterone on the heart can more clearly define the mechanisms of its modulation on the pathogenesis of DbCM. Although testosterone is known to modulate immune cell activity, there is a paucity of work investigating the impact of testosterone on cardiomyocyte-specific inflammation, in the context of diabetes or otherwise.[79] One study found that testosterone increased levels of TNF-α in cardiomyocytes; however, the mechanisms responsible for this were not investigated.[80]

TF is a protein involved in the stimulation of the coagulation cascade. It is expressed at high levels in cardiomyocytes and is also believed to be involved in the pathology of DbCM.[81] STZ-injected mice expressing reduced levels of tissue factor (TF) had attenuated *IL-6*, and *TLR4* expression compared with STZ-injected mice expressing normal levels of *TF*.[82] Given the potential inhibitory role of testosterone on TF through androgen-dependent tissue factor pathway inhibitor-regulating protein (ADTPR), inhibition of TF represents one way in which testosterone may inhibit inflammation associated with hyperglycemia.[83,84] *ADTPR* expression is increased through testosterone/AR binding to its promoter region. Although expression of this protein is confirmed in the heart, no research has been done on its direct impact on cardiomyocytes.[83,84]

Cardiomyocytes cultured in high glucose had an increase in *NF-κB* expression.[85] Similarly, inhibition of NF-κB in STZ-injected rats led to reduced TNF-α and IL-2.[86] Knockdown of *TLR4* expression using siRNA in STZ-injected mice led to decreased cardiac levels of mRNAs encoding TNF-α, IL-1, ICAM-1, and VCAM-1.[87] AGEs have been shown to increase NF-κB activation and *TNF-α* expression in cardiomyocytes.[71] Knockout of *RAGE* reduced the expression of *NF-κB*, *TNF-α*, and *IL-6* in left ventricular homogenates of mice fed a high-fat diet.[70] Because cardiomyocytes were not isolated, the extent to which cardiomyocyte-specific inflammatory activity is reduced by *RAGE* blockade remains uncertain. AGE can also elicit inflammation through TLR binding. TLR4 was found to bind AGE, which led to increased NF-κB activity and increased IL-6 and TNF-α mRNA. These changes were blunted by inhibition of TLR4 binding to AGE.[68] Overall, these results suggest that hyperglycemia and hyperlipidemia can trigger NF-κB signaling in cardiomyocytes, leading to the expression of proinflammatory cytokines.

Diabetes/hyperglycemia can also induce inflammation through activation of the nucleotide-binding oligomerization domain like receptor (NLR) pyrin domain containing 3 (NLRP3) inflammasome. This leads to the secretion of IL-1β and IL-18, which enhance inflammation.[88,89] Cardiomyocytes cultured in high glucose displayed increased cell death, increased *NLRP3* expression, and secretion of IL-1β, TNF-α, and IL-18.[74,75] In STZ-injected and high-fat diet—fed rats, increased IL-1β expression and *NLRP3* expression were noted relative to controls.[75]

Cytokines released by inflammation may also have an effect on cardiomyocytes. Whether IL-6 induces or protects against apoptosis in cardiomyocytes is controversial.[90,91] Testosterone was found to blunt the increase in cleaved caspase 3 in response to IL-6 administration, but other apoptosis-associated proteins were unchanged by its administration.[91] TNF-α and IL-1β are observed to induce apoptosis in cardiomyocytes, whereas TNF-α, IL-1β, IL-6, and IL-18 were shown to promote hypertrophy.[56] There is also evidence that inflammation can lead to reduced testosterone levels.[92,93]

Protein kinase C (PKC) is a serine/threonine kinase.[94] Protein levels of PKC-β1, -β2, and -α, as well as total PKC activity, are increased in failing human hearts compared with healthy controls.[95] Cardiomyocytes cultured in high glucose had increased expression of *PKC-α* and *PKC-β2*, and nuclear translocation of the protein. Inhibition of PKC reduced *NF-κB* expression and protein nuclear translocation, and decreased TNF-α levels.[85] This suggests that PKC is upstream of NF-κB. Because testosterone-AR signaling increases levels of PKCδ, the effects on inflammation in the context of diabetic models are worthy of investigation.[96]

Oxidative stress can directly cause inflammation in cardiomyocytes, and resolving oxidative stress inhibited NLRP3 activation in cardiomyocytes cultured in high glucose.[74,75] Similarly, HO-1 appears to be protective against inflammation. Systemic overexpression of *HO-1* in STZ-injected mice ablated cardiac inflammation.[47] Stimulation of *HO-1* expression by testosterone may therefore attenuate inflammation in DbCM.

Hypertrophy

Cardiac hypertrophy involves the increase in size of individual cardiomyocytes leading to an increase in overall myocardial mass. This often occurs in response to

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mechanical pressure and can be a nonpathological process associated with stimuli such as exercise or pregnancy. However, if hypertrophy interferes with cardiac function due to being prolonged or excessive in nature, it is known as pathological cardiac hypertrophy.[97]

Epidemiological evidence suggests that hypertrophy is associated with diabetes, independent of hypertension.[4] In fact, DbCM is first characterized by left ventricular hypertrophy leading to diastolic dysfunction.[6,20] Hypertrophy is noted in cardiomyocytes cultured in high glucose and animal models of diabetes, suggesting that hyperglycemia directly contributes to hypertrophy.[98] There is much evidence to suggest that testosterone promotes the development of myocardial hypertrophy.[96,99–104]

Mammalian target of rapamycin (mTOR) is a serine/threonine kinase, involved in increasing protein synthesis. In the case of cardiomyocytes, mTOR regulates hypertrophy in response to pressure.[8,105] Administration of testosterone to isolated cells or animals was shown to increase cardiomyocyte hypertrophy through mTOR activation.[99,101] However, there is a paucity of data on the potential role of mTOR in DbCM.[106] mTOR inhibition was shown to attenuate hypertrophy in animal models of diabetes, but whether mTOR activity is actually elevated in the context of diabetes may depend on the type of model used.[107,108]

Nuclear factor of activated T-cells (NFAT) is a transcription factor that is known to promote hypertrophy,[109] and cardiomyocytes cultured in high glucose have increased nuclear NFAT levels. However, the dependency of hypertrophy in cells cultured in high glucose on NFAT signaling was not investigated.[110] Testosterone has been shown to enhance NFAT activity in cardiomyocytes.[104]

Glycogen synthase kinase-3β (GSK-3β) is a protein kinase associated with anti-hypertrophic activity.[111] GSK-3β phosphorylates serine residues on NFAT, promoting its export from the nucleus.[112] Testosterone was found to promote the phosphorylation/deactivation of GSK-3β, through a PI3K/Akt-dependent pathway. In fact, the hypertrophic effects of testosterone appear to depend on GSK-3β phosphorylation.[104] Conversely, inhibition of GSK-3β in STZ-injected rats diminished hypertrophy.[86] However, the chemical used to inhibit the GSK-3β pathway (meisoindigo), also inhibited Wnt/β-catenin, which may have been responsible for the antihypertrophic effects.[86]

Myocyte enhancer factor−2 (MEF2) is a transcription factor that has been associated with myocardial hypertrophy.[113] Testosterone-induced hypertrophy in rats and isolated cardiomyocytes was dependent on MEF2 nuclear translocation and signaling. Such effects were dependent on AR-binding.[114]

Calmodulin-dependent protein kinase II (CaMKII) is known to mediate cardiac pathology, including DbCM.[115,116] CaMKII can bind and phosphorylate histone-deacetylases (HDACs) present in the heart including HDAC4. This results in the shuttling of HDAC4 out of the nucleus. HDAC4 is involved in suppression of MEF2 signaling, so its removal from the nucleus can increase MEF2 transcriptional activity.[114,117] Testosterone-associated hypertrophy is dependent on CaMKII activity.[114] Increased nuclear NFAT and decreased nuclear HDAC4 were noted in a rat model of diabetes.[118] Although this suggests that diabetes may activate these pro-hypertrophic pathways, no hypertrophy was noted in this model. Overall, more work must be done to investigate the role of NFAT and HDAC4 in DbCM, especially in models presenting with hypertrophy. Similarly, whether HDAC4 mediates the dependency of testosterone-NFAT signaling on CaMKII should be further investigated.

Hypertrophy in cells exposed to elevated concentrations of glucose was shown to be dependent on PKC activation.[98] Inhibition of PKC-β prevented hypertrophy in diabetic animal models.[86,119] Elevations in maternal testosterone were shown to increase cardiac hypertrophy in rat offspring, concomitant with high cardiac levels of PKCδ, dependent on AR-testosterone signaling.[96] TF was found to be increased in the hearts of diabetic mice. Reduced expression of TF was linked to attenuated hypertrophy in STZ-injected mice.[82] Because TF is linked to hypertrophy in models of diabetes, ADTPR may have an antihypertrophic role.

Increased Na+/H+ exchanger 1 (NHE1) activity was noted in hearts from mouse models of type 2 diabetes.[120] *NHE1* overexpression in mice was associated with increased nuclear NFAT and decreased nuclear HDAC4, as well as cardiac hypertrophy, whereas inhibition of NHE1 activity reduced cardiac hypertrophy.[121–123] It is not known whether testosterone regulates NHE1 activity in cardiomyocytes.

Inhibition of XO activity led to decreased cardiomyocyte hypertrophy in mice fed a high fat diet.[54] Testosterone administered to late-gestational rats increases cardiac ADA and XO activity.[55] However, hypertrophy was not directly measured in this study.

Inflammatory pathways may also lead to hypertrophy in DbCM, although crosstalk with testosterone has not been explored. Inhibition of NF-κB attenuated hypertrophy in high-glucose−cultured cardiomyocytes or in the myocardium of STZ-injected mice.[86,98] AGEs were found to increase hypertrophy in cardiomyocytes and knockout of the receptor for AGEs (*RAGE*) led to decreased cardiac hypertrophy in mice fed a high-fat diet.[70] Silencing of *TLR4* in STZ-injected mice decreased hypertrophy, providing further evidence for the inflammation−hypertrophy theory in diabetic animal models.[87]

Oxidative stress is also believed to be a cause of myocardial hypertrophy.[23] Overexpression of the antioxidant *HO-1* in cardiomyocytes protected against hypertrophy.[62] Similarly, PKC-β inhibition in STZ-injected rats led to improvements in both myocardial hypertrophy and oxidative stress.[124] Myocardial hypertrophy in the presence of hyperglycemia may be due to inflammation or oxidative stress, so preventing oxidative stress in diabetic hearts may decrease hypertrophic remodeling.

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Table 1 Summary of Animal Models Used to Study Diabetic Cardiomyopathy

Animal model	Observed effects	Effect of testosterone
STZ-injection	Increased oxidative stress[29,32,43,47,53,60,67,71,124,125]	Decreased oxidative stress[32]
	Reduced antioxidant defense[32,67]	Increased antioxidant defense[32]
	Increased inflammation[29,43,47,60,67,68,71,74,75,86,87,125,126]	
	Increased fibrosis[29,43,47,53,68,86,87,125,126]	
	Increased hypertrophy[86,87,124–126]	
(mRen-2)27 transgenic rats injected with STZ	Increased oxidative stress[119]	NR
	Increased fibrosis[119]	
	Increased hypertrophy[119]	
High fat/fructose diet (Western diet)	Increased oxidative stress[29,54,70,108]	NR
	Increased inflammation[29,70,74,75]	
	Increased fibrosis[29,54]	
	Increased hypertrophy[54,70,108]	
Obese *db/db* mice	Increased oxidative stress[65,108]	NR
	Increased hypertrophy[108]	
Hyperamylinemia	Increased hypertrophic signaling[118]	NR
GK (Goto-Kakizaki) rats	Increased hypertrophy[120]	NR

NR, XXX. Q12

Fibrosis

Cardiac fibrosis has been noted in patients and animal models of diabetes. Collagen I and II deposits and increased tissue growth factor (TGF)-β are noted in both the right and left ventricles of patients and experimental models.[76] AGEs, oxidative stress, and inflammation have been found to stimulate myocardial fibrosis.[76] The role of increased myocardial fibroblast activity in the production of fibrosis in DbCM is well-established, but there is less data focused on the role of cardiomyocytes.[76]

One theory is that the replacement of dead cardiomyocytes with fibrous tissue leads to increased myocardial fibrosis in DbCM.[76] Cardiomyocyte apoptosis is largely triggered by oxidative stress and inflammation, as reviewed earlier. Additionally, hyperglycemia may cause the release of cytokines which stimulate other cells to produce fibres.[76] Cardiomyocytes cultured in elevated concentration of glucose or glucose and high fatty acid exhibit increased expression of *TGF-β*, which is known to lead to fibroblast activation.[125–127] IL-6 is also known to increase *TGF-β* expression, suggesting that inflammation may be responsible for this effect.[91] Testosterone supplementation was shown to be insufficient to prevent IL-6–induced increased *TGF-β* expression, but this does not rule out a protective role against fibrosis through other pathways.[91] It is also possible that the cardiomyocytes are secreting collagen and actin, directly causing myocardial fibrosis. Both hyperglycemia or hyperglycemia/fatty acids have been shown to lead to increased *collagen I, III*, and *IV* and *α-actin* expression in cardiomyocytes.[125–128]

The inhibition of Nrf2 signaling is believed to be responsible for the induction of fibrosis in high-glucose environments. As mentioned previously, early stages of diabetes are believed to entail increased *Nrf2* expression, which is reversed as the disease progresses.[43] Knockdown of *Nrf2* was associated with increased expression of *α-smooth muscle actin* and *collagen I.*[128] Unfortunately, the impact of testosterone on Nrf2 signaling is not known.

Similarly, NF-κB signaling may be responsible for fibrosis in DbCM. Inhibition of NF-κB in STZ-injected rats decreased myocardial fibrosis.[86] GSK-3β inhibition in STZ-injected mice reduced myocardial fibrosis.[86] AGE exposure was shown to increase cardiomyocyte expression of collagen I and TGF-β1. These cells also displayed greater NF-κB and GSK-3β activation, as well as decreased Nrf2 activation.[71]

In addition to inflammation and oxidative stress, PKC may be involved in fibrosis, because inhibition of PKC-β in STZ-injected rats led to decreased collagen I compared with controls.[86,119] The putative protective effect of Nrf2 and detrimental roles of GSK-3β, NF-κB, and PKC in the stimulation of cardiomyocyte fibrosis is in concordance with their effects on inflammation and oxidative stress pathways. Evidence from animal models suggests that oxidative stress causes myocardial fibrosis to which cardiomyocytes may contribute.[76]

Although evidence suggests that testosterone promotes myocardial fibrosis, no studies have investigated the direct impact of testosterone on the induction of fibrosis in cardiomyocytes.[79,96] Similarly, the extent to which cardiomyocyte matrix protein expression and profibrotic cytokine expression actually increases myocardial fibrosis in an *in vivo* context cannot be inferred from studies using isolated cardiomyocytes.

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Conclusion and Future Directions

Overall, there is limited work directly investigating the impact of testosterone in animal models of diabetes. Given the presence of sex differences in the development of DbCM, it is important to investigate the potential impact of sex hormones such as testosterone on the development of this condition.[23] Because previous evidence suggests that the impact of testosterone is dependent upon glycemic status, it is difficult to infer the role of testosterone in DbCM based on studies in nondiabetic animals[124,129] (Table 1). Therefore, the influence of testosterone on the development of DbCM must be investigated by examining how testosterone administration directly impacts this pathology.

Disclosure Statement

None declared.

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