Endocrine, *vol. 25, no. 2, 73–83, November 2004* 0969–711X/04/25:73–83/$25.00

REVIEW

Diabetic Cardiomyopathy

Do Women Differ From Men?

Jun Ren and Asli F. Ceylan-Isik

Division of Pharmaceutical Sciences and Center for Cardiovascular Research and Alternative Medicine, University of Wyoming, Laramie, WY 82071

Although many aspects of cardiovascular disease are similar between women and men, it is becoming increasingly obvious that there are significant differences as well. Premenopausal women ususally have a lower risk of cardiovascular diseases than age-matched men and postmenopausal women. However, the "female advantage" disappears once women are affilicted with diabetes mellitus. Heart diseases are twice as common in diabetic men and five times as common in diabetic women. It is believed that differences in sex hormones and intrinsic myocardial and endothelial functions between men and women may be responsible for this female "advantage" and "disadvantage" in normal and diabetic conditions. Most experimental and clinical studies on diabetes only included male subjects and failed to address this important gender difference in diabetic heart complications. Although female hearts may be better tolerated to stress (such as ischemia) insults than their male counterparts, female sex hormone such as estrogen may interact with certain risk factors under diabetes which may compromise the overall cardiac function. The benefit versus risk of estrogen replacement therapy on cardiac function and overall cardiovascular health in diabetes remains controversial. This review will focus on gender-related difference in diabetic heart complication—diabetic cardiomyopathy—and if gender differences in intrinsic myocardial contraction, polyol pathway metabolism, and advanced glycation endproduct formation and other neuroendocrinal regulatory mechanisms to the heart may contribute to disparity in diabetic cardiomyopathy between men and women.

Key Words: Diabetes; diabetic cardiomyopathy; cardiac; gender.

Received August 2, 2004; Revised October 17, 2004; Accepted October 21, 2004.

Author to whom all correspondence and reprint requests should be addressed: Dr. Jun Ren, Associate Professor, Division of Pharmaceutical Sciences and Center for Cardiovascular Research and Alternative Medicine, University of Wyoming College of Health Sciences, Laramie, WY 82071. E-mail: jren@uwyo.edu

Introduction

Cardiovascular diseases are among the leading causes of morbidity and mortality in patients with diabetes mellitus. The incidence of diabetes is expected to double over the next decade owing to the sedentary lifestyle and an ever growing cluster of pre-diabetic syndromes including syndrome X, obesity, and insulin resistance *(1)*. Almost all of these metabolic disturbances are considered major risk factors for development of heart dysfunction and congestive heart failure *(2–6)*. Sustained diabetes of either type leads to deterioration of heart function known as diabetic cardiomyopathy, which occurs independent of macro- and microvascular diseases *(7)*. Diastolic dysfunction is the most prominent mechanical defect in diabetic cardiomyopathy and is characterized by decreased compliance and slower rates of myocardial relaxation *(7–9)*. Both systolic and diastolic dysfunctions have been characterized as prolonged duration of contraction and relaxation, reduced velocity of contraction and relaxation, and depressed myocardial contractility in whole heart, tissue, and isolated ventricular myocytes from both diabetic patients and experimental animals *(7–9)*. Although the pathogenesis of diabetic cardiomyopathy has not been precisely described, several mechanisms have been speculated including reduced energy production due to decrease in mitochondrial respiration and pyruvate dehydrogenase activity, accumulation of free radical species, malfunction of cardiac contractile and intracellular Ca^{2+} regulatory proteins such as myosin, titin, sarco(endo)plasmic reticulum Ca^{2+}–ATPase (SERCA), phospholamban, and Na^{+}–Ca^{2+} exchanger *(7,10–12)*. The increased risk of diabetic cardiomyopathy and other heart complications warrants stringent and aggressive treatment against hyperglycemia, hyperinsulinemia, dyslipidemia, and oxidant damage. The most commonly used therapeutic regimes in diabetic patients with heart dysfunctions encompass angiotensin-converting enzyme (ACE) inhibitors, angiotensin-receptor antagonists, digoxin, diuretics, β-blockers, Ca^{2+} antagonists, and spironolactone. The insulin-sensitizing agents such as thiazolidinediones and peroxisome proliferators-activated receptors (PPARs) agonists are recommended in treatment

of diabetes over insulin-secretion-enhancing agents to avoid hyperinsulinemia and insulin resistance. In addition to pharmacological interventions, primary care for diabetic patients also includes lifestyle modifications such as smoking cessation, weight control, exercise, and dietary restriction *(6)*. Routine and moderate exercise may reduce blood glucose, blood pressure, body weight and body fat, and improve insulin sensitivity, lipid profile, as well as the overall cardiac pumping function in diabetic individuals *(6)*. Nevertheless, without the precise knowledge of the mechanism(s) of action for this devastating myopathic state, most of the therapeutic strategies are far from optimization to manage the morbidity and mortality of diabetic cardiomyopathy effectively.

One of the rather interesting and puzzling clinical observations is that diabetes can cancel off the normal "female advantage" in cardiovascular function. Without diabetes, women usually display a much lower incidence of heart disease prior to menopause but only lose this "female" advantage following menopause, suggesting that ovarian hormones, primarily estrogen, may play a pivotal role in the reduced risk for heart disease *(13)*. However, heart diseases are twice as common in diabetic men and five times more common in diabetic women when compared with the general population *(3)*. Gender-related differences in myocardial function and tolerance to stress such as ischemia reperfusion injury have been documented *(14–17)*. Women usually display higher peak and end-systolic ventricular pressures when compared to men *(18)*. Sex difference in intrinsic myocardial contractile function including duration and maximal velocity of contraction and relaxation has been well recognized *(14–17,19)*. These intrinsic myocardial or hemodynamic differences between men and women, along with female sex hormones (estrogen, progesterone) and male sex hormone testosterone, are believed to be contribute to gender-related disparities in cardiac morbidity and mortality under noth normal and diabetic conditions. Clinical and experimental evidence indicated that estrogen replacement therapy may ameliorate cardiac risk, although this notion was challenged recently *(5,20–22)*. While estrogen replacement may benefit cardiac contractile action, blood lipid profile, vascular resistance, and oxidative stress *(22)*, recent clinical trials [e.g., Heart and Estrogen/Progestin Replacement Study (HERS), Women's Ischemia Syndrome Evaluation (WISE), and Women's Health Initiative (WHI)] have brought us some unpredicted or even surprising findings regarding the impact of estrogen replacement on heart function *(23)*. Results of these clinical trials demonstrated that estrogen replacement therapy does not provide cardiovascular benefits in women with established heart disease. The general consensus is that women should not consider estrogen replacement without any known benefits of estrogen replacement therapy for cardiovascular function. As awareness of women's health issues continues to rise, it is pertinent and important to discuss the diabetic heart complications especially diabetic cardiomyopathy in women. Up-to-date, only limited information is available toward understanding human diabetic cardiomyopathy in both men and women, as many studies only included males as experimental subjects. This review will summarize the gender-related myocardial contractile function and metabolic alteration (such as polyol pathway) under diabetes and involvement of estrogen and other neurohormonal factors in gender-related differences in diabetes-related heart complication with a special focus on diabetic cardiomyopathy. Because certain factors such as male sex hormone testosterone, age, and peripheral vascular activity may also participate in the manifestation of diabetes-associated cardiac contractile dysfunction, the gender-asociated disparities in diabetic heart complications appear to be more complex than originally thought and require more intensive research.

Table 1
Major Forms of Heart Diseases
in Men and Women With or Without Diabetes Mellitus

Patient groups	Most commonly seen heart diseases in adult men and women with the highest morbidity and mortality *(95–98)*
Men (non-diabetic)	Coronary calcification, coronary artery disease, myocardial infarction, atrial fibrilation, angina
Men (diabetic)	Coronary calcification, coronary artery disease, myocardial infarction, coronary heart disease (worse than non diabetic men), diastolic abnormality, autonomic neuropathy, congestive heart failure (twice the frequency of non-diabetic men)
Women (non-diabetic)	Myocardial infarction (better prognosis than diabetic women)
Women (diabetic)	Coronary calcification, coronary artery disease, myocardial infarction (worse than men), coronary heart disease (better prognosis than men); diastolic abonormality, autonomic neuropathy, congestive heart failure (fivefold the frequency of non-diabetic women)

Gender and Myocardial Electromechanical Function in Diabetes

Although men have a higher mortality rate, more women than men die from heart failure *(24)*. Just as it is in men, heart disease is also the major cause of mortality and disability in women. Table 1 summarizes the major forms of heart diseases in non-diabetic and diabetic men and women. Coronary heart diseases, heart attacks, and heart muscle (cardiomyopathies) and valvular heart diseases in the postmenopausal years are common and serious problems for women. A recent survey on gender factor in heart failure, often the endpoint of cardiomyopathy, including over 230 reports suggested that prevalence for heart failure is similar in both genders, although men display a higher incidence

of heart failure. Women are usually older when first being diagnosed with heart failure and survive longer after that *(25)*. Some forms of heart disease may be more difficult to be recognized in women than in men. For example, cardiac diastolic dysfunction is usually more severe in women than men *(25)*. It is therefore impossible and inaccurate to arbitrarily apply the knowledge obtained from men to women. Clinically, diabetic cardiomyopathy is manifested as impaired systolic, but mainly diastolic, function *(7)*. In experimental diabetes, myocardial mechanical properties are significantly altered at whole heart, multi- and single cellular levels, characterized by prolonged contraction and relaxation and a marked reduction of relaxation velocity *(7,10)*. Epidemiological studies revealed that premenopausal diabetic women display a comparable incidence of cardiac complications compared to age-matched male patients. However, a significant increase in mortality is seen in diabetic women after menopause compared to age-matched diabetic men *(5)*. Because most women spend at least one-third of their lives in an "estrogen-free" or postmenopausal state, the abrupt increase in cardiac mortality in postmenopasual diabetic women is believed to contribute to the overt higher risk of heart diseases in women when associated with diabetes. Because estrogen replacement has been demonstarted to favorably influence several risk factors in post-menopausal diabetic patients including dyslipidemia, hypertension, and endothelial dysfunction *(5)*, it may be natural to speculate that the state of female sex hormones, primarily estrogen, may be a significant factor in the ultimate cardiac health in women associated with diabetes. However, the obvious difference in myocardial electromechanical properties between men and women seems to have drawn more attention recently in an effort to explain the gender difference in diabetic cardiomyopathy or diabetic heart muscle diseases.

Impaired systolic and diastolic ventricular contractile functions are both characteristic of diabetic cardiomyopathy *(8)*. Myocardium and ventricular myocytes from diabetic hearts exhibit reduced contractility, prolonged duration, and slowed rate of contraction and relaxation *(7,8,10–12,16,26)*, which are correlated with prolonged action potentials *(27,28)*, depressed Ca^{2+} reuptake by sarcoplasmic reticulum (SR) *(29)*, isozyme switch from α- to β-myosin heavy chain (MHC) *(30)*, alteration in cardiac troponin T expression and troponin I phosphorylation *(31,32)*. Finding from our laboratory reveals that isolated ventricular myocytes from OVE26 type 1 diabetic mice possesses significantly reduced peak contraction amplitude and maximum velocity of contraction/relaxation, as well as prolonged duration of contraction/relaxation compared to those from wild-type FVB mice *(19)*. These mechanical defects are paralleled with abnormal intracellular Ca^{2+} homeostasis (mainly shown as prolonged intracellular Ca^{2+} transient decay) in myocytes from OVE26 diabetic mice. Non-diabetic female mouse myocytes exhibited a weaker contractile function compared to the age-matched normal male counterparts. Interestingly, this gender-specific difference was "cancelled off" by diabetes. The young female OVE26 mice exhibited notably better-preserved contractile function, whereas cardiac contraction of the older female was poorly maintained compared to the age-matched male mice, indicating the existence of "female advantage" in young but not old female diabetic mice *(19)*. Our very recent data in streptozotocin (STZ)-induced diabetes in FVB mice (2- to 3-mo-old) revealed similar findings. Weight-matched (22–24 g) male and female FVB mice were made diabetic with a single ip injection of STZ (200 mg/kg) and maintained for 2 wk. Mechanical evulation of ventricular myocyte contractile function indicated that female myocytes exhibit weaker peak shortening (PS) and maximal velocity of shortening/relengthening (± *dL/dt*) associated with longer contraction (TPS) duration. Diabetes depressed PS and ± *dL/dt*, and prolonged TPS as well as relaxation duration (TR90) in male but not female myocytes (Fig. 1), supporting the notion of "female advantage" in young diabetic state. Interestingly, when the same experiment (same mouse age, weight, and STZ treatment) was repeated using transgenic mice with cardiac overexpression of antioxidant metallothionein (approx 10-fold), neither gender nor STZ (200 mg/kg i.p. maintained for 2 wk) effect was seen (Fig. 2). This is supported by our earlier report that metallothionein protects against diabetic cardiomyopathy in OVE26 type 1 diabetes *(33)*. The fact that metallothionein transgene abolished gender differences in myocyte function under both non-diabetic and diabetic conditions suggests that disparity in intrinsic antioxidant capacity may play a role in gender-related differences in myocardial function. Like metallothionein, estrogen is a known antioxidant *(34)* and may provide certain protection on myocardial function under diabetic insult. Consistently, our earlier study using ventricular papillary muscles also suggested that diabetes may ablate the gender-specific difference in myocardial function *(16)*. Diabetes significantly reduced the maximal velocity of tension rise and decline in male but not female myocardium although there was no significant difference in contraction or relaxation parameters between the two genders under diabetic state *(16)*. However, one of our interesting findings indicated that the gender-specific difference in young diabetic mice is "interrupted" when aging is taken into account. Myocytes from young (premenopasual) female diabetic animals possess better-preserved contractile function compared to age-matched male diabetic mice. However, this "female advantage" disappeared in older (presumably postmenopausal stage) female diabetic mice. In essence, older female OVE26 mice displayed a markedly reduced maximum velocity of contraction and relaxation, and a prolonged duration of shortening and relengthening compared to older male counterparts *(19)*.

Epidemiological and experimental studies have demonstarted distinct differences in myocardial contractile function between men and women. Myocytes from male subjects

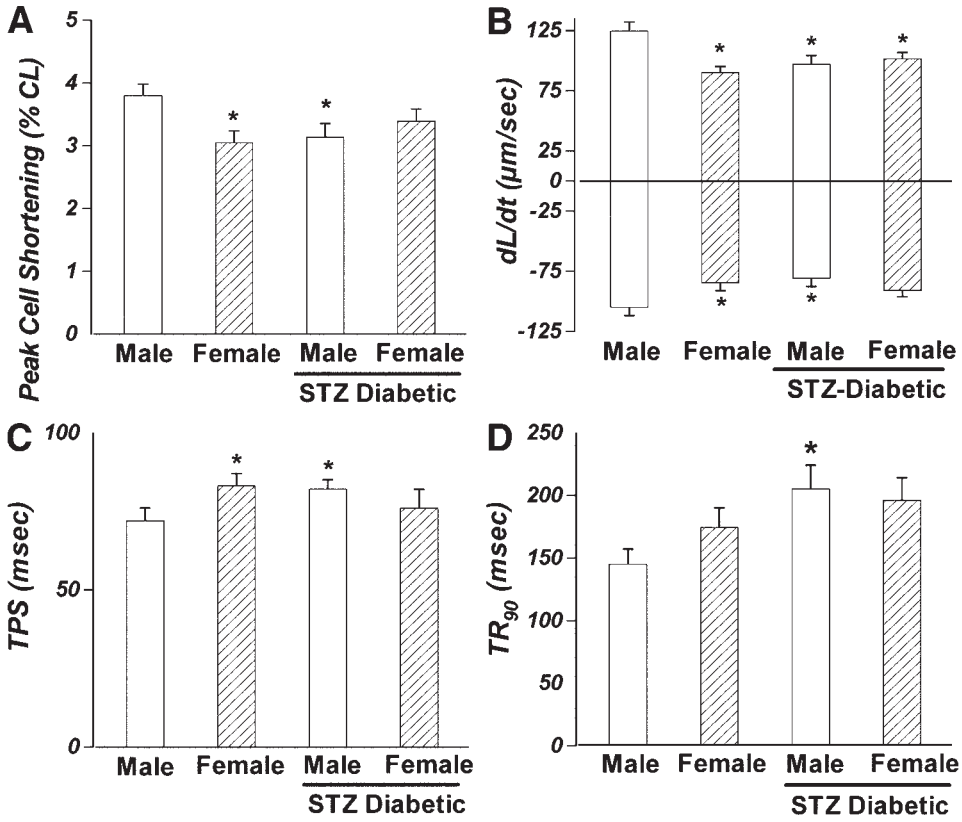


Fig. l. Contractile properties of ventricular myocytes from male and female, normal and streptozotocin (STZ, 200 mg/kg, single ip injection, maintained for 2 wk)-induced diabetic mice. Weight-matched (22–24 g, 3–4 mo of age) albino FVB mice of both genders were used. The elevation of blood glucose induced by STZ was equal in both male and female diabetic mice. Mechanical function of myocytes was evaluated with an IonOptix MyoCam system, while the myocytes were stimulated to contract at a frequency of 0.5 Hz. (**A**) Peak shortening (PS as % of resting cell length). (**B**) Maximal velocity of shortening/relengthening (± *dL/dt*). (**C**) Time-to-peak shortening (TPS). (**D**) Time-to-90% relengthening (TR_{90}). Mean ± SEM, $n = 81–88$ myocytes from five mice per group. *$p < 0.05$ male control group.

undergo a greater degree of hypertrophy and postnatal growth than their age-matched female. Although the magnitude of myocardial contraction is comparable between male and female, contraction and relaxation durations are usually shorter and the maximum velocities of tension development and decline are markedly faster in female myocardium *(14)*. These results were supported by our finding in bilateral ovariectomized female rats. We noted depressed maximal velocity of contraction/relaxation, prolonged duration of contraction/relaxation in ventricular myocytes from ovariectomized rat hearts associated with reduced peak contractility, slowed intracellular Ca^{2+} clearing, and elevated intracellular Ca^{2+} levels. These mechanical defects may be prevented by estrogen replacement *(35)*. Our data suggested that ovariectomy did not alter levels of key cardiac Ca^{2+} regulating proteins including SERCA, phospholamban, and total protein kinase B (PKB)/Akt, but significantly reduced PKB/Akt activation (pAkt), and ratios of pAkt/Akt or SERCA 2a/phospholamban. The alterations in protein expression were restored by estrogen replacement *(35)*. Ovariectomy during pre- and postpubertal periods has been shown to lead to decreased cardiac output, peak systolic pressure, and ejection fraction associated with reduced myosin ATPase activity and MHC isoenzyme shift (V1 to V3) *(14)*, which can be prevented by estrogen replacement therapy *(35,36)*. Somewhat inconsistent with the findings in human and rat models, unloaded ventricular myocytes from female mice exhibits reduced peak contraction amplitude and maximum rate of contraction/relaxation, and a longer duration of relaxation as compared to male counterparts *(19)*. This, nevertheless, was in line with an earlier study that displays a weaker contractile function in female hearts *(37)* (also shown in Fig. 1). Therefore, caution should be taken when addressing gender-related myocardial electromechanical function from different species. In addition, the age of the experimental objects may also affect the outcome of the gender-related myocardial eletromechanical function. Our data suggested that gender difference in myocardial contractile

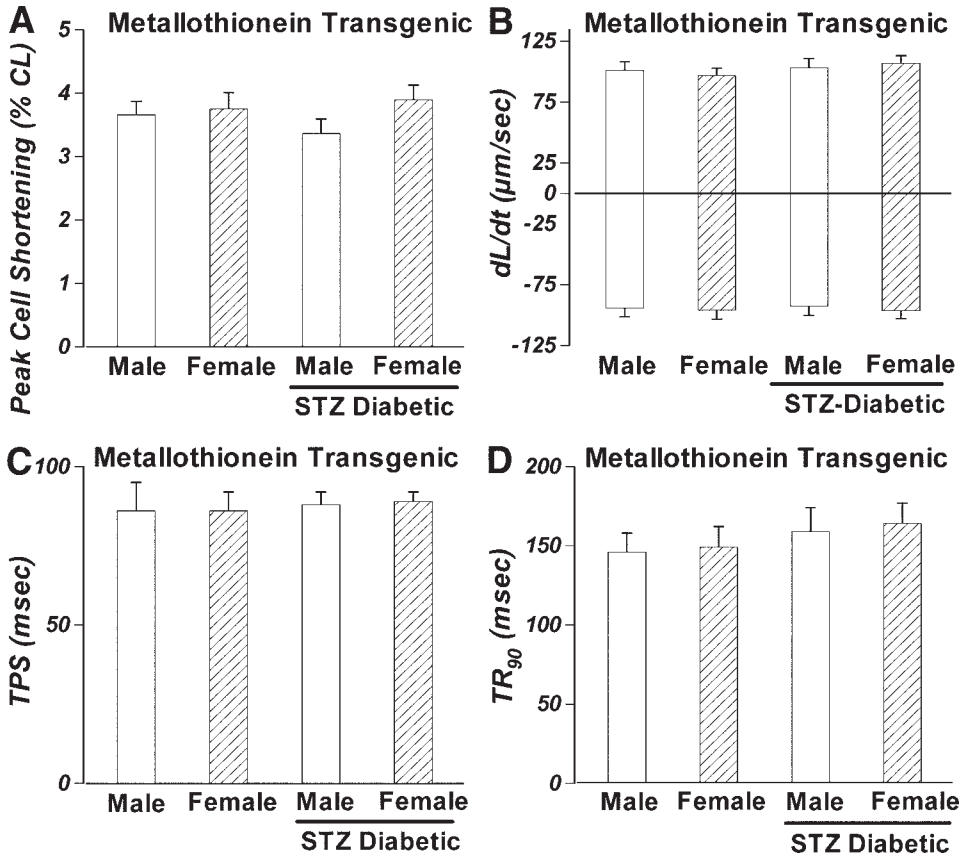


Fig. 2. Contractile properties of ventricular myocytes from cardiac metallothionein (approx tenfold) overexpression transgenic, normal and streptozotocin (STZ, 200 mg/kg, single ip injection, maintained for 2 wk)-induced diabetic mice, Weight-matched (22–24 g, 3 mo of age) metallothionein transgenic mice of both genders were used. The elevation of blood glucose induced by STZ was equal in both male and female diabetic mice. Mechanical function of myocytes was evaluated with an lonOptix MyoCam system while the myocytes were stimulated to contract at a frequency of 0.5 Hz. (**A**) Peak shortening (PS as % of resting cell length). (**B**) Maximal velocity of shortening/relengthening (± *dL/dt*). (**C**) Time-to-peak shortening (TPS). (**D**) Time-to-90% relengthening (TR_{90}). Mean ± SEM, n = 65–70 myocytes from metallothionein mice per group.

function may be significantly augmented in older mice *(19)*. It is suggested that age-related mechanical differences may be due to discrepant expression of cardiac contractile proteins (α- and β-MHC), Ca^{2+} regulating proteins (e.g., SERCA and phospholamban), or events regulating cardiac action potential configuration (e.g., membrane ion channel conductance) *(35,38–40)*. Paradoxically, many of these proteins have been demonstrated to display compromised function or protein expression in the heart following short-term or sustained diabetes mellitus *(7,12,40–42)*.

Up-to-date, the cellular and molecular basis of intrinsic factors contributing to gender disparity of diabetic cardiomyopathy is essentially unrevealed. In a recent study of heart failure, which is a common endpoint consequence for diabetic cardiomyopathy, the SR Ca^{2+} cycling function, protein levels of SERCA, phospholamban, and calsequestrin, and phosphorylation of phospholamban were examined in failing and donor myocardia from both genders *(43)*. The results showed that the protein levels of phospholamban and calsequestrin were not altered, whereas that of SERCA was significantly reduced equally in failing hearts of both genders. Phosphorylation of phospholamban and myocardial cAMP content were both attenuated in failing myocardia. V_{max} and EC50 for SERCA Ca^{2+} uptake function were decreased and increased, respectively, in failing myocardia. The reduction in phospholamban phosphorylation and elevation in EC50 of SERCA function were specific to male but not female failing hearts *(43)*. These data suggested that difference in myocardial Ca^{2+} cycling between men and women may play a significant role in the gender-disparity of heart failure. Such gender-related differences in Ca^{2+} cycling proteins may be of significance in the elucidation of gender difference in diabetic cardiomyopathy because impaired intracellular Ca^{2+} homostasis as a result of impaired Ca^{2+} cycling proteins is believed to contribute to the pathogenesis of this devastating diabetic myopathy *(7,8,44)*.

One of the most crucial electromechnical alterations in diabetic cardiomyopathy is the prolonged action potential

duration in diabetic or high glucose–treated ventricular myocytes *(27)*. It has been demonstrated that reduction in repolarizing outward K^+ currents is responsible for the prolonged reploariztion phase (phase III) of cardiac action potential *(42)*. There are no significant differences in action potential between genders for 3- and 9-mo-old non-diabetic rats *(38)*. However, both transient and delayed-rectifying outward K^+ currents were smaller in ventricular myocytes from non-diabetic female rats compared to male counterparts. Diabetes significantly attenuated both transient and delayed-rectifying outward K^+ currents in myocytes from males but only significantly suppressed the delayed-rectifying outward K^+ current without affecting the transient outward K^+ current in females. Nevertheless, the degree of diabetes-induced reduction of delayed-rectifying outward K^+ currents was much smaller in females than in males *(40)*. Inhibitors of either ACE or endothelin-converting enzyme (ECE) significantly enhanced K^+ currents in myocytes from male diabetic rats whereas exhibiting no effect in cells from female diabetic rats. However, inhibition of either angiotensin II or endothelin formation augmented both K^+ currents in ventricular myocytes from ovariectomized diabetic females, which can be reverted by estrogen replacement *(40)*. These results collectively depicted that (1) a gender difference in outgoing repolarizing K^+ currents exists under diabetes; (2) an autocrine modulation of K^+ currents by renin–angiotensin and endothelin systems may be compromised in female diabetic rats largely due to estrogen. Cell capacitance of myocytes from female hearts was found to be smaller than that from the age-matched male myocytes *(45,46)*. Reduced transient outward of K^+ currents in females *(40)* and, consequently, increase in action potential duration may predispose women to more severe forms of prolongation of myocardial relaxation and diabetic cardiomyopathy. This seems to be supported by the clinical observation that cardiac diastolic dysfunction is usually more severe in women than men *(25)*. On the other side of the coin, the suppressed autocrine modulation of outward K^+ currents by renin–angiotensin and endothelin-1 systems in the "estrogen-intact" female diabetic rats may serve to compensate for the smaller outward K^+ current or other intracellular Ca^{2+} cycling processes such as Ca^{2+} channel. The fact that diabetes only significantly inhibited delayed-rectifying outward K^+ current without affecting the transient outward K^+ current in "estrogen-intact" females *(40)* also offer an explanation for cancellation of diabetes against gender difference in K^+ currents under non-diabetic conditions. In addition to transient or delayed-rectifying outward K^+ currents, estrogen has been shown to affect other components of action potential configuration. Ovariectomy was shown to either upregulate *(47)* or down-regulate *(48)* the L-type Ca^{2+} channel density. Recent studies showed that ovariectomy-induced modulation of gene expression of the large-conductance Ca^{2+}-activated K^+ channel and L-type voltage-gated Ca^{2+} channel in vascular smooth muscle cells was completely prevented in ovariectomized rats receiving chronic treatment with estrogen *(49)*.

Gender Difference in Neuroregulatory System of the Heart

Diabetes has been shown to upregulate the activity of several neuroendocrinological systems including renin–angiotensin, adrenergic, endothelin, and nitric oxide (NO) systems *(50,51)*. The autocrine/paracrine action of angiotensin II has been demonstrated to contribute to suppress repolarizing outward K^+ currents therefore leading to prolonged action potential duration in cardiomyocytes from both type 1 and type 2 diabetic models *(52)*. This is consistent with the observation that inhibition of the angiotensin-converting enzyme or blockade of angiotensin II receptors produced augmentation of K^+ currents due to enhanced synthesis of channel proteins *(53)*. However, inhibition of angiotensin II or endothelin-1 formation using ACE or ECE inhibitors augmented outward K^+ currents in ventricular myocytes from male diabetic or ovariectomized female diabetic animals but not in "estrogen-intact" female diabetic or ovariectomized/estrogen re-supplemented female diabetic animals *(40)*. This finding clearly indicated existence of gender-difference in angiotensin II or endothelin-1 response in diabetes-induced cardiac electromechanical alteration. β-Adrenergic stimulation leads to cAMP accumulation, activation of protein kinase A (PKA), and, subsequently, phosphorylation of cellular proteins such as L-type Ca^{2+} channel. A lower density (or cell capacity) of L-type Ca^{2+} channels in female myocytes may contribute to smaller inotropic response to β-adrenergic stimulation. Diabetes mellitus is usually associated with reduced or blunted cardiac β-adrenergic response probably due to down-regulation of β-adrenergic receptors in ventricular myocytes *(11,40)*. On the other hand, high sensitivity β-adrenergic system or overexpression of β-adrenergic receptors, if sustained, is usually related to cardiac electromechanical dysfunction and heart failure *(54)*. Thus, the lower β-adrenergic receptor density in myocardium in females may explain lower mortality in women than men with symptomatic heart failure *(55)* although it did not seem to favor diabetic cardiac complications in women where β-adrenergic response is already abnormal. The reduced phosphorylation of sarcolemmal L-type Ca^{2+} channel in females may lead to reduce SR Ca^{2+} release and re-uptake due to the Ca^{2+}-induced Ca^{2+} release mechanism, resulting a less efficient SERCA function in females *(56)*. Meanwhile, SR Ca^{2+} release may become less sensitive to trigger Ca^{2+} sparks through Ca^{2+} channel due to the reduced β-adrenergic stimuli in ventricular myocytes from females, making the female ventricular myocytes more prone to diabetes-induced electromechanical dysfunction.

Recently, attention has been drawn toward the role of NO and its reactive compounds in the development of dia-

betes cardiovascular complications. NO, an extremely reactive gas with chemical properties of free radicals, is synthesized by a variety of cell types including cardiac myocytes. NO participates in a cascade of pathophysiological processes including diabetes when formed in excess or in the presence of other pro-oxidants *(57,58)*. Enhanced endogenous production of NO or addition of NO donors has been documented to result in compromised cardiac function and enhanced apoptosis *(57)*, illustrating the potential toxic properties of NO. NO and O_2^- react rapidly (second-order rate constant = 6.7×109 *M*/s) to generate peroxynitrite ($ONOO^-$), which rapidly decomposes to highly oxidant species such as nitronium ion (NO_2^+). Overproduction of the free radical NO with subsequent development of local oxidative stress has been proposed to be one of the significant pathophysiological mechanisms for diabetes-induced cardiovascular dysfunctions. Blockade of increased NO production by simultaneous administration of NOS inhibitors or scavengers for NO and $ONOO^-$ have been shown to suppress the hyperglycemia or diabetes-induced cardiac electromechanical dysfunctions *(51,58)*. Not surprisingly, both endothelial NOS (eNOS) and neuronal NOS (nNOS) are subject to regulation by estrogen, which may explain some myocardial alterations during pregnancy and certain pathophysiological conditions *(59)*. The gender difference in the bioavailability of NO due to ovarian estrogen and possibly other factors such as difference in certain lipoproteins (LDL and HDL) contributes to the low cardiac risk in women *(34)*. However, the higher basal NO production, eNOS protein expression, and NOS activity in female cardiac myocytes from our study *(60)* also conferred female heart at higher risk of accumulating NO-associated reactive free radicals and therefore is more predispose to diabetes-induced cardiac electromechanical dysfunction. As a double-edge sword, NO is responsible for both cardiac protection and insult, further study is warranted to understanding the precise role of NO in female gender-related regulation of myocardial contractile function under both normal and diabetic conditions.

Last but certainly not least, leptin, a hormone linking adiposity and central nervous circuits to reduce appetite and enhance energy expenditure, may also contribute to the gender disparity in diabetic heart complications. Serum levels of leptin are significantly higher in women than men with equivalent body fat mass because women have much greater subcutaneous adipose tissue mass relative to omental adipose tissue than men *(61)*. The production of leptin is speculated to be under the influence of female sex hormone estrogen *(61)*. Leptin is known to increase the overall sympathetic nerve activity, facilitate glucose utilization, and improve insulin sensitivity. In addition, leptin is capable of regulating (depressing) cardiac and vascular contractility through a NO-dependent mechanism. However, elevated plasma leptin level has been demonstrated to correlate with insulin resistance, obesity, hyperlipidemia, and diabetes, independent of total adiposity. Elevated plasma leptin level, subsequently tissue leptin resistance, are considered independent risk factors for the development of cardiovascular disease *(62)*. The higher serum levels of leptin and the close association between leptin and diabetes, as well as the fact that leptin directly depresses cardiac contractile function has prompted our speculation that leptin may contribute to gender difference in cardiac function. Nevertheless, how much, and if any, influence of higher serum leptin levels to cardiac electromechanical function in women with diabetes deserves in depth investigation.

Gender Difference in Myofilament Ca^{2+} Responsiveness

One of the rationales for the pathogenesis of cardiac mechanical dysfunction is the compromised myofilament responsiveness to Ca^{2+} in ventricular myocytes under diabetes *(10, 63)*. Coincidentally, there is also an overt gender-related difference in myofilament Ca^{2+} responsiveness that is believed to contribute to the disparity of myocardial contractile function between men and women. Female myocardia seem to possess a lower myofilament Ca^{2+} sensitivity than those from men, making the female myocardia more predisposed to diabetes-induced reduction in myofilament Ca^{2+} sensitivity. Ovariectomy has been shown to increase the left ventricular myofilament Ca^{2+} sensitivity *(64,65)*. The estrogen, or estrogen deficiency to be exact, elicited influence on myofilament Ca^{2+} sensitivity may occur in the absence of significant change in the maximum force development *(66)*. This may explain why peak myocardial tension development was not affected by either diabetes or gender whereas other indices exhibited differential alteration by diabetes between males and females *(16)*.

Gender, Sex Hormones, and Polyol Pathway

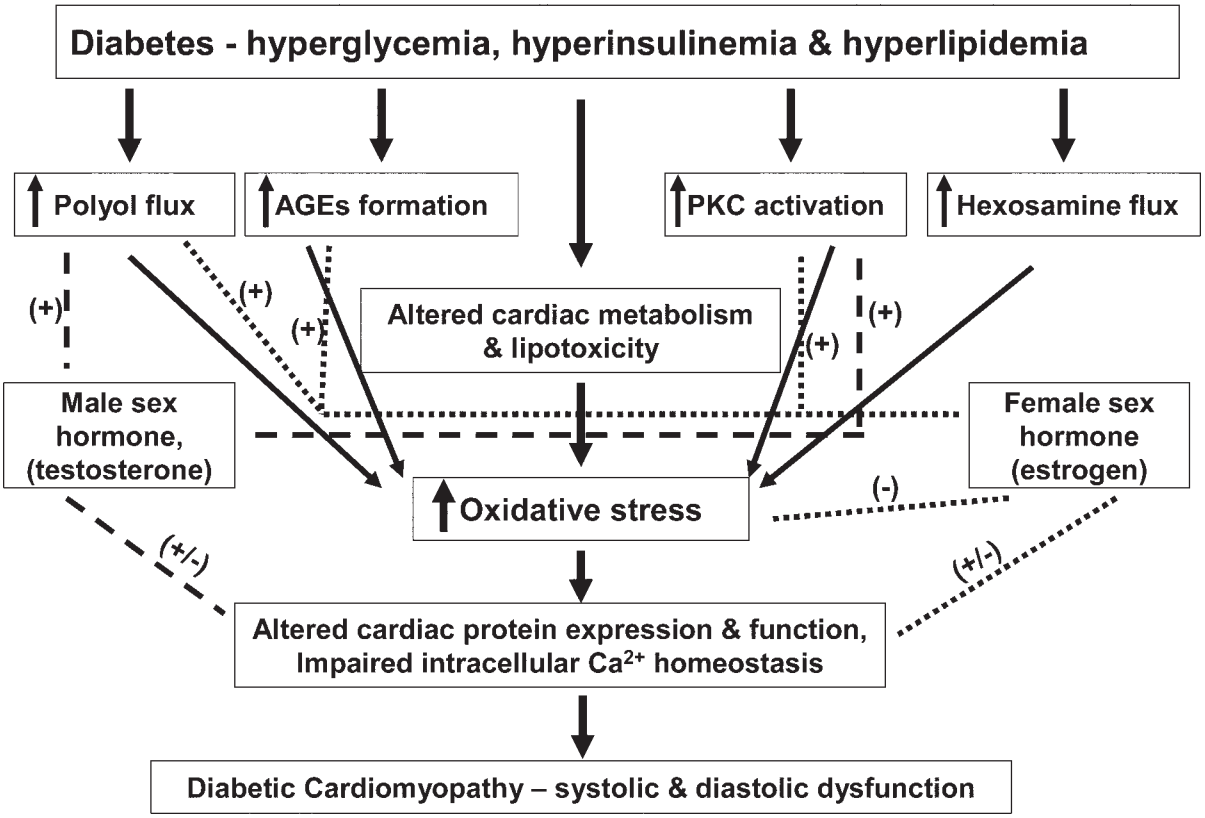
In addition to alteration in cardiac contractile proteins and intracellular Ca^{2+} homeostasis due to hyperglycemia, hyperinsulinemia (insulin resistance), dyslipidemia, and accumulation of cytotoxic free radical, several other factors may also play a role in the gender-related disparity of diabetic cardiomyopathy. A total of four main hypotheses regarding how hyperglycemia causes diabetic complications have been postulated based on an ample of experimental and clinical evidence, some of which were based on results using specific inhibitors of respective signaling pathways. The four main pathways are increased polyol pathway flux; increased advanced glycation endproducts (AGEs) formation; activation of protein kinase C (PKC) isoforms; and increased hexosamine flux *(67,68)*. One key mechanism proposed for diabetic complication is enhanced glucose utilization by aldose reductase (AR) *(68)*. AR is the first enzyme in the polyol pathway, which catalyses the NADPH-depen-

dent reduction of carbonyl compounds, including glucose. Glucose is converted to sorbitol by AR via oxidation of NADPH, and sorbitol is then oxidized by sorbitol dehydrogenase (SDH) to fructose at the cost of NAD^+. Normally, AR has a low affinity for glucose in a non-diabetic, low glucose state. However its affinity for glucose is much higher in the diabetic state, which converts glucose to sorbitol, with resultant decreases in NADPH *(69)*. An increased AR activity under diabetes is often correlated with increased $NADH/NAD^+$ *(69,70)*. Levels of sorbitol and fructose were approx ninefold higher and $NADH/NAD^+$ was approx fourfold higher in diabetic hearts than those of non-diabetic ones *(70)*. Experiments demonstrated that cytosolic $NADH/NAD^+$ was reduced by inhibition of SDH or AR *(69–71)*. The increased $NADH/NAD^+$ ratio leads to inhibition of the enzyme glyceraldehydes-3-phosphate dehydrogenase (GAPDH) and accumulation of triose phosphate *(72)*, which promotes the formation of AGEs and diacylglycerol (DAG) and activation of PKC *(68)*. Evidence suggested that AR may interact with sex hormones and thus be responsible for a polyol pathway–associated difference in diabetic complications between genders *(73)*. Diabetes-induced changes in polyol metabolism was shown to be abolished by castration in STZ-treated rats, suggesting an interaction between this pathway and androgens *(74)*. It was speculated that sequence consensus between the AR promoter region and an androgen response element may contribute to the interaction between AR and androgen *(73)*. Genetic polymorphisms in this promoter region are closely associated with enhanced risk of diabetic complications such as diabetic nephropathy *(73)*, although little is known on cardiomyopathy. On the other hand, estrogen may enhance the mRNA expression of AR in endothelial cells *(75)*, which would be expected to link to a higher risk of diabetic complications. However, it seems that certain indirect effect of estrogen on AR may effectively "offset" its own upregulation on AR. It is believed that NO can maintain AR in an inactive state and that this "lock" is relieved by diabetes *(76)*. Thus, increasing NO availability, commonly offered by estrogen *(34,59, 60)*, may be a useful strategy for inhibiting the polyol pathway and preventing the development of diabetes complications. It is worthy mentioning that contributions of the polyol pathway in diabetes are often tissue and species specific and may not fully explain the pathogenesis of all forms of diabetic complications *(68)*.

Gender Impact on AGEs and Diabetic Cardimyopathy

Results from our lab have demonstrated that hyperglycemia alone may contribute to the onset of diabetic cardiomyopathy *(77,78)*, and the high glucose–mediated cardiac excitation–contraction coupling defect is possibly due to enhanced protein glycosylation *(79)*. On the other hand, depressed diastolic function in the heart of diabetic patients has been related to increased concentration of circulating AGEs *(80–82)*. The Maillard or Browning reaction between sugars and proteins leads to the formation of chemical modification and cross-links in proteins, known as AGEs *(67, 68)*. These sugar-derived modifications have been suggested to play a central role in the pathogenesis of diabetic complication and aging-associated complications *(83)*. It is suggested that hyperglycemia contributes to diabetic complication through formation of AGEs, which ultimately resulted in modified function of macromolecule due to crosslink formation between functional proteins and AGEs *(67,68)*. Through interaction with receptors, AGEs are capable of eliciting oxygen free radical generation. Consequently, increased cellular oxidative stress leads to the activation of the free radical sensitive transcription factor NF-κB thus prompts the expression of NF-κB regulated genes involved in the development of atherosclerosis and apoptosis *(84)*. Pathological changes as a result of AGE accumulation in diabetes may be summarized in three general mechanisms *(67,68)*. First, AGEs alter signal transduction pathways involving ligands on extracellular matrix. Second, AGEs alter the level of soluble signals such as cytokines, hormones, and oxygen free radicals through interactions with AGE-specific cellular receptors. Third, intercellular AGEs formation by glucose, fructose, and more highly reactive metabolic pathway intermediates can directly alter protein function in target tissue. Consistent with the AGE hypothesis, the AGE inhibitors, such as aminoguanidine and pyridoxamine, inhibit the formation of AGEs in various proteins in vitro and in collagen in vivo and retard the development of diabetic complication in animal models *(67,68)*. Alternately, ALT-711, a stable 4,5-dimethylthiazolium derivative of N-phenacylthiazolium bromide and a novel class of AGE crosslink "breaker" *(85)*, may be considered as a promising clinical candidate in reversing the deterioration of cardiovascular function, which ultimately results in hypertension, diabetic cardiomyopathy, and congestive heart failure *(86–88)*. Aminoguanidine and pyridoxamine, inhibitors of AGE formation, can block the development of cardiovascular complications of diabetes in animal models *(89)*. Benfotiamine, a lipid-soluble thiamine derivative, was shown to effectively prevent activation of three major pathways involved in hyperglycemia-induced tissue damage: the hexoamine pathway, the intracellular AGE formation pathway, and the diacylglycerol-PKC pathway *(90)*. In addition to the intrinsic myocardial electromechanical difference between men and women "theory," possible gender disparities or interaction between sex hormones and one of the above mentioned signaling pathways (e.g., polyol pathway, AGEs, PKC, and hexosamine flux) may also contribute to the gender difference in the manifestation of diabetic cardio-myopathy.

AGEs are known to deteriorate various cell functions through binding to the receptor for AGEs (RAGE). Bind-



Scheme 1. Potential mechanisms of action of diabetic cardiomyopathy and influence of sex hormones. The (+) and (–) signs indicate "stimulation" and "inhibition" of an effect, respectively. (+/–) indicates mixed action (stimulation or inhibition) depending on the protein of interest. Absence of dashed line between the sex hormone boxes and an "effect" box indicates lack of effect or evidence for any effect.

ing of AGEs to RAGE may trigger oxidative modifications of critical Ca^{2+} regulatory proteins such as SERCA, leading to impaired function of SERCA and ultimately diabetic cardiomyopathy. The observations that AGE–RAGE engagement altered intracellular Ca^{2+} homeostasis, but without quantitative changes in the Ca^{2+} transport protein SERCA, imply the possibility that impaired intracellular Ca^{2+} homeostasis may be mediated through oxidative modification of Ca^{2+} regulatory proteins by AGEs *(91)*. Although we are not aware of any gender-related difference in AGE formation, estrogen itself has been shown to up-regulate mRNA and protein levels of RAGE and activate the RAGE gene through NFκB and SP-1, resulting in enhanced AGE–RAGE interactions *(92)*. This observation suggests that women with functional ovary may possess a higher AGE–RAGE interaction compared with men and thus may exacerbate the diabetic heart complications. In addition to the upregulation of RAGE, estrogen and progesterone have also been shown to stimulate the expression of PKC isozymes in a tissue-specific manner (although report in heart has not been seen) *(93)*. Whether there is any gender-related difference in hexosamine pathway or interaction between sex hormone such as estrogen with hexosamine pathway has not been elucidated. A scheme is provided to illustrate the likely contributing mechanisms for diabetic cardiomyopathy and how they may be affected by sex hormones. Further investigations are warranted to better illustrate the role of gender in each of the four contributing pathways to diabetic complication proposed by Brownlee and colleagues *(67,68)*.

Conclusion

The scenario behind gender-related difference in the propensity and clincal manifestation of diabetic cardiomyopathy and cardiac health may be attributed to the intrinsic myocardial electromechanical properties, sex hormones (estrogen, progesterone, androgen), and other neurohormonal factors between men and women. Female sex hormones such as estrogen and progesterone has undoubtly participated in the "on and off switch" of certain contributing factors in the pathogenesis of diabetic cardiomyopathy such as cardiac outward K^+ channels. However, the male sex hormone androgen also plays a critical role in the regulation of cardiovascular function not only in men but also in women. In postmenopausal women, ovary becomes an important source of androgens, which produces unopposed (by estrogen) action on cardiovascular system (such as hypertension) after menopause *(94)*. In addition, the presentation, investigation, and

treatment of diabetic heart complications in women and the specific management and assessment of overall cardaiac health in diabetic women should be addressed and deserve intensive further investigation.

Acknowledgments

The work in the Ren laboratory has been supported in part by National Institute of Health, American Diabetes Association, American Heart Association (Northland and Pacific Mountain Affiliates), Max Baer Heart Foundation, and University of Wyoming Faculty Grant-in-Aid.

References

1. Strandberg, T. E. and Salomaa, V. (2000). *Nutr. Metab. Cardiovasc. Dis.* **10,** 239–246.
2. Charpentier, G., Genes, N., Vaur, L., et al. (2003). *Diabetes Metab.* **29,** 152–158.
3. Garcia, M. J., McNamara, P. M., Gordon, T., and Kannel, W. B. (1974). *Diabetes* **23,** 105–111.
4. Pradhan, A. D., Skerrett, P. J., and Manson, J. E. (2002). *J. Cardiovasc. Risk* **9,** 323–330.
5. Sowers, J. R. (1998). *Arch. Intern. Med.* **158,** 617–621.
6. Li, S., Culver, B., and Ren, J. (2003). *Pharmacol. Res.* **48,** 127–132.
7. Fein, F. S. and Sonnenblick, E. H. (1994). *Cardiovasc. Drugs Ther.* **8,** 65–73.
8. Ren, J. and Davidoff, A. J. (1997). *Am. J. Physiol.* **272,** H148–H158.
9. Ren, J., Walsh, M. F., Hamaty, M., Sowers, J. R., and Brown, R. A. (1998). *Am. J. Physiol.* **275,** H823–H830.
10. Ren, J. and Bode, A. M. (2000). *Am. J. Physiol. Heart Circ. Physiol.* **279,** H238–H244.
11. Norby, F. L., Aberle, N. S., Kajstura, J., Anversa, P., and Ren, J. (2004). *J. Endocrinol.* **180,** 175–182.
12. Norby, F. L., Wold, L. E., Duan, J., Hintz, K. K., and Ren, J. (2002). *Am. J. Physiol. Endocrinol. Metab.* **283,** E658–E666.
13. Farhat, M. Y., Lavigne, M. C., and Ramwell, P. W. (1996). *FASEB J.* **10,** 615–624.
14. Capasso, J. M., Remily, R. M., Smith, R. H., and Sonnenblick, E. H. (1983). *Basic Res. Cardiol.* **78,** 156–171.
15. Skafar, D. F., Xu, R., Morales, J., Ram, J., and Sowers J. R. (1997). *J. Clin. Endocrinol. Metab.* **82,** 3913–3918.
16. Brown, R. A., Anthony, M. J., Petrovski, P., and Ren, J. (2001). *Cardiovasc. Toxicol.* **1,** 35–42.
17. Brown, R. A., Walsh, M. F., and Ren, J. (2001). *Endocr. Res.* **27,** 399–408.
18. Hayward, C. S., Kalnins, W. V., and Kelly, R. P. (2001). *Cardiovasc. Res.* **49,** 340–350.
19. Zhang, X., Ye, G., Duan, J., Chen, A. F., and Ren, J. (2003). *Endocr. Res.* **29,** 227–236.
20. Enserink, M. (2002). *Science* **297,** 755–757.
21. Ho, J. E. and Mosca, L. (2002). *Curr. Atheroscler. Rep.* **4,** 387–395.
22. Subbiah, M. T. (2002). *Braz. J. Med. Biol. Res.* **35,** 271–276.
23. Mosca, L., Collins, P., Herrington, D. M., et al. (2001). *Circulation* **104,** 499–503.
24. Hoppe, B. L. and Hermann, D. D. (2003). *Curr. Cardiol. Rep.* **5,** 193–199.
25. Stromberg, A. and Martensson, J. (2003). *Eur. J. Cardiovasc. Nurs.* **2,** 7–18.
26. Ren, J. (2000). *Cardiovasc. Res.* **46,** 162–171.
27. Ren, J., Gintant, G. A., Miller, R. E., and Davidoff, A. J. (1997). *Am. J. Physiol.* **273,** H2876–H2883.
28. Jourdon, P. and Feuvray, D. (1993). *J. Physiol.* **470,** 411–429.
29. Ganguly, P. K., Pierce, G. N., Dhalla, K. S., and Dhalla, N. S. (1983). *Am. J. Physiol.* **244,** E528–E535.
30. Dillmann, W. H. (1980). *Diabetes* **29,** 579–582.
31. Liu, X., Takeda, N., and Dhalla, N. S. (1996). *Biochim. Biophys. Acta* **1316,** 78–84.
32. Akella, A. B., Ding, X. L., Cheng, R., and Gulati, J. (1995). *Circ. Res.* **76,** 600–606.
33. Ye, G., Metreveli, N. S., Ren, J., and Epstein, P. N. (2003). *Diabetes* **52,** 777–783.
34. Rossouw, J. E. (2002). *Cardiovasc. Res.* **53,** 550–557.
35. Ren, J., Hintz, K. K., Roughead, Z. K., et al. (2003). *Am. J. Physiol. Heart Circ. Physiol.* **284,** H1800–H1807.
36. Scheuer, J., Malhotra, A., Schaible, T. F., and Capasso, J. (1987). *Circ. Res.* **61,** 12–19.
37. Luchner, A., Brockel, U., Muscholl, M., et al. (2002). *Cardiovasc. Res.* **53,** 720–727.
38. Leblanc, N., Chartier, D., Gosselin, H., and Rouleau, J. L. (1998). *J. Physiol.* **511(Pt 2),** 533–548.
39. Rosenkranz-Weiss, P., Tomek, R. J., Mathew, J., and Eghbali, M. (1994). *J. Mol. Cell. Cardiol.* **26,** 261–270.
40. Shimoni, Y. and Liu, X. F. (2003). *J. Physiol.* **550,** 401–412.
41. Duan, J., Zhang, H. Y., Adkins, S. D., et al. (2003). *Am. J. Physiol. Endocrinol. Metab.* **284,** E366–E376.
42. Shimoni, Y., Firek, L., Severson, D., and Giles, W. (1994). *Circ. Res.* **74,** 620–628.
43. Dash, R., Frank, K. F., Carr, A. N., Moravec, C. S., and Kranias, E. G. (2001). *J. Mol. Cell. Cardiol.* **33,** 1345–1353.
44. Ren, J., Dominguez, L. J., Sowers, J. R., and Davidoff, A. J. (1996). *Diabetes* **45,** 1822–1825.
45. Wahler, G. M. (1992). *Am. J. Physiol.* **262,** C1266–C1272.
46. Walker, K. E., Lakatta, E. G., and Houser, S. R. (1993). *Cardiovasc. Res.* **27,** 1968–1977.
47. Patterson, E., Ma, L., Szabo, B., Robinson, C. P., and Thadani, U. (1998). *J. Pharmacol. Exp. Ther.* **284,** 586–591.
48. Bowling, N., Bloomquist, W. E., Cohen, M. L., et al. (1997). *J. Pharmacol. Exp. Ther.* **281,** 218–225.
49. Tsang, S. Y., Yao, X., Wong, C. M., Chan, F. L., Chen, Z. Y., and Huang, Y. (2004). *Eur. J. Pharmacol.* **483,** 155–162.
50. Sechi, L. A., Griffin, C. A., and Schambelan, M. (1994). *Diabetes* **43,** 1180–1184.
51. Haluzik, M. and Nedvidkova, J. (2000). *Physiol. Res.* **49(Suppl 1),** S37–S42.
52. Shimoni, Y. (2001). *J. Physiol.* **537,** 83–92.
53. Shimoni, Y. and Liu, X. F. (2003). *Am. J. Physiol. Heart Circ. Physiol.* **284,** H1168–H1181.
54. Keys, J. R. and Koch, W. J. (2004). *Recent Prog. Horm. Res.* **59,** 13–30.
55. Pijna, I. L. and Buchter, C. (2003). *Cardiol. Rev.* **11,** 337–344.
56. Eisner, D. A., Choi, H. S., Diaz, M. E., O'Neill, S. C., and Trafford, A. W. (2000). *Circ. Res.* **87,** 1087–1094.
57. Kojda, G. and Harrison, D. (1999). *Cardiovasc. Res.* **43,** 562–571.
58. Esberg, L. B. and Ren, J. (2003). *Diabetologia* **46,** 1419–1427.
59. Weiner, C. P., Lizasoain, I., Baylis, S. A., Knowles, R. G., Charles, I. G., and Moncada, S. (1994). *Proc. Natl. Acad. Sci. USA* **91,** 5212–5216.
60. Esberg, L. B., Zhang, X., Scott, G. I., Culver, B., and Ren, J. (2004). *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **138,** 141–146.
61. Considine, R. V. (2001). *Rev. Endocr. Metab. Disord.* **2,** 357–363.
62. Ren, J. (2004). *J. Endocrinol.* **181,** 1–10.
63. Hofmann, P. A., Menon, V., and Gannaway, K. F. (1995). *Am. J. Physiol.* **269,** H1656–H1663.
64. Wattanapermpool, J., Riabroy, T., and Preawnim, S. (2000). *Life Sci.* **66,** 533–543.
65. Wattanapermpool, J. (1998). *Life Sci.* **63,** 955–964.
66. Wattanapermpool, J. and Reiser, P. J. (1999). *Am. J. Physiol.* **277,** H467–H473.

67. Brownlee, M. (1995). *Clin. Invest. Med.* **18,** 275–281.
68. Brownlee, M. (2001). *Nature* **414,** 813–820.
69. Ramasamy, R. (2003). *Curr. Drug Targets* **4,** 625–632.
70. Ramasamy, R., Oates, P. J., and Schaefer, S. (1997). *Diabetes* **46,** 292–300.
71. Hwang, Y. C., Bakr, S., Ellery, C. A., Oates, P. J., and Ramasamy, R. (2003). *FASEB J.* **17,** 2331–2333.
72. Williamson, J. R., Chang, K., Frangos, M., et al. (1993). *Diabetes* **42,** 801–813.
73. Lane, P. H. (2002). *Am. J. Physiol. Renal. Physiol.* **283,** F589–F600.
74. Williamson, J. R., Rowold, E., Chang, K., et al. (1986). *Diabetes* **35,** 20–27.
75. Villablanca, A. C., Lewis, K. A., and Rutledge, J. C. (2002). *J. Appl. Physiol.* **92,** 1064–1073.
76. Chandra, D., Jackson, E. B., Ramana, K. V., Kelley, R., Srivastava, S. K., and Bhatnagar, A. (2002). *Diabetes* **51,** 3095–3101.
77. Davidoff, A. J. and Ren, J. (1997). *Am. J. Physiol.* **272,** H159–H167.
78. Ren, J., Dominguez, L. J., Sowers, J. R., and Davidoff, A. J. (1999). *Diabetes* **48,** 2059–2065.
79. Ren, J., Gintant, G. A., Miller, R. E., and Davidoff, A. J. (1997). *Am. J. Physiol.* **273,** H2876–H2883.
80. Berg, T. J., Snorgaard, O., Faber, J., et al. (1999). *Diabetes Care* **22,** 1186–1190.
81. Kilhovd, B. K., Berg, T. J., Birkeland, K. I., Thorsby, P., and Hanssen, K. F. (1999). *Diabetes Care* **22,** 1543–1548.
82. Asano, G., Takashi, E., Ishiwata, T., et al. (2003). *J. Nippon Med. Sch.* **70,** 384–392.
83. Bierhaus, A., Hofmann, M. A., Ziegler, R., and Nawroth, P. P. (1998). *Cardiovasc. Res.* **37,** 586–600.
84. Vlassara, H. (2001). *Diabetes Metab. Res. Rev.* **17,** 436–443.
85. Vasan, S., Zhang, X., Zhang, X., et al. (1996). *Nature* **382,** 275–278.
86. Asif, M., Egan, J., Vasan, S., et al. (2000). *Proc. Natl. Acad. Sci. USA* **97,** 2809–2813.
87. Kass, D. A., Shapiro, E. P., Kawaguchi, M., et al. (2001). *Circulation* **104,** 1464–1470.
88. Liu, J., Masurekar, M. R., Vatner, D. E., et al. (2003). *Am. J. Physiol. Heart Circ. Physiol.* **285,** H2587–H2591.
89. Baynes, J. W. and Thorpe, S. R. (2000). *Free Radic. Biol. Med.* **28,** 1708–1716.
90. Hammes, H. P., Du, X., Edelstein, D., et al. (2003). *Nat. Med.* **9,** 294–299.
91. Petrova, R., Yamamoto, Y., Muraki, K., et al. (2002). *J. Mol. Cell. Cardiol.* **34,** 1425–1431.
92. Tanaka, N., Yonekura, H., Yamagishi, S., Fujimori, H., Yamamoto, Y., and Yamamoto, H. (2000). *J. Biol. Chem.* **275,** 25781–25790.
93. Ruzycky, A. L. and Kulick, A. (1996). *Eur. J. Pharmacol.* **313,** 257–263.
94. Thompson, J. and Khalil, R. A. (2003). *Clin. Exp. Pharmacol. Physiol.* **30,** 1–15.
95. Raggi, P., Shaw, L. J., Berman, D. S., and Callister, T. Q. (2004). *J. Womens Health (Larchmt.)* **13,** 273–283.
96. Lansky, A. J., Mehran, R., Dangas, G., et al. (2004). *Am. J. Cardiol.* **93,** 916–919.
97. Timoteo, A. T., Ferreira, J., Aguiar, C., et al. (2003). *Rev. Port Cardiol.* **22,** 367–374.
98. Aronson, D. and Rayfield, E. J. (1998). In: *Textbook of cardiovascular medicine*. 9th ed. Topol, E. J. (ed.). Lippincott–Raven: Philadelphia, PA, pp. 171–194.