Sex Differences in Renal and Cardiovascular Function: Physiology and Pathophysiology

Hypersensitivity of myofilament response to Ca\(^{2+}\) in association with maladaptation of estrogen-deficient heart under diabetes complication

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Thawornkaiwong A, Pantharanontaga J, Wattanapermpool J. Hypersensitivity of myofilament response to Ca\(^{2+}\) in association with maladaptation of estrogen-deficient heart under diabetes complication. Am J Physiol Regul Integr Comp Physiol 292: R844–R851, 2007. First published October 12, 2006; doi:10.1152/ajpregu.00365.2006.—The amelioration of cardioprotective effect of estrogen in diabetes suggests potential interactive action of estrogen and insulin on myofilament activation. We compared Ca\(^{2+}\)-dependent Mg\(^{2+}\)-ATPase activity of isolated myofibrillar preparations from hearts of sham and 10-wk ovariectomized rats with or without simultaneous 8 wk-induction of diabetes and from diabetic-ovariectomized rats with estrogen and/or insulin supplementation. Similar magnitude of suppressed maximum myofibrillar ATPase activity was demonstrated in ovariectomized, diabetic, and diabetic-ovariectomized rat hearts. Such suppressed activity and the relative suppression in α-myosin heavy chain level in ovariectomy combined with diabetes could be completely restored by estrogen and insulin supplementation. Conversely, the myofilament Ca\(^{2+}\) hypersensitivity detected only in the ovariectomized but not diabetic group was also observed in diabetic-ovariectomized rats, which was restored upon estrogen supplementation. Binding kinetics of β\(_1\)-adrenergic receptors and immunoblots of β\(_1\)-adrenoceptors as well as heat shock 72 (HSP72) were analyzed to determine the association of changes in receptors and HSP72 to that of the myofilament response to Ca\(^{2+}\). The amount of β\(_1\)-adrenoceptors significantly increased concomitant with Ca\(^{2+}\) hypersensitivity of the myofilament, without differences in the receptor binding affinity among the groups. In contrast, changes in HSP72 paralleled that of maximum myofibrillar ATPase activity. These results indicate that hypersensitivity of cardiac myofilament to Ca\(^{2+}\) is specifically induced in ovariectomized rats even under diabetes complication and that alterations in the expression of β\(_1\)-adrenoceptors may, in part, play a mechanistic role underlying the cardioprotective effects of estrogen that act together with Ca\(^{2+}\) hypersensitivity of the myofilament in determining the gender difference in cardiac activation.

The well-recognized gender difference in the incidence of cardiovascular disease has led to a number of studies on the influence of female sex hormones on cardiac contractile activation. Suppressed maximum myofibrillar ATPase activity, myofibrillar Ca\(^{2+}\) hypersensitivity, and a significant shift in myosin heavy chain (MHC) toward the β-MHC isoform have been demonstrated in ovariectomized (OVX) rat hearts (40, 41). Upregulation of β\(_1\)-adrenoceptors, which may partly underlie changes in the myofilament Ca\(^{2+}\) activation, was also detected in OVX heart (37). The similarity of Ca\(^{2+}\) hypersensitivity detected in OVX hearts (40, 41) to that in cardiomyopathic hearts (20, 42, 43) previously reported supports the beneficial role of estrogen (E\(_2\)) in the myocardial activation.

Surprisingly, the cardioprotective effect of estrogen on myocardial function seems to be overcome by diabetes (18, 22, 36). The morbidity and mortality of cardiovascular diseases in diabetic patients appear to be increased in females compared with age-matched males. These gender differences in the incidence of heart disease suggest that deprivation of estrogen and insulin induces interactive effects on the cardiac myofilament response to Ca\(^{2+}\). This notion is indirectly supported by the observation of an additive effect of estrogen deficiency and diabetes on bone mineral density in diabetic-ovariectomized rats (14). Moreover, deficiency of estrogen increases the severity of renal disease in a diabetic rat model in which estrogen replacement is renoprotective by improving renal function and pathology associated with diabetes (29). Despite these reports on the combined effects of estrogen and insulin on various organs, their interactive effects on the cardiac myofilament response to Ca\(^{2+}\) and the underlying mechanism remain unknown.

Alterations in the regulatory effect of β\(_1\)-adrenergic stimulation and the protective effect of heat shock proteins (HSP) appear to play important roles in the effects of estrogen and insulin on the cardiac myofilament response to Ca\(^{2+}\). Stimulation of β\(_1\)-adrenergic receptors plays a physiologically significant role in enhancing cardiac contractility through modification of Ca\(^{2+}\) flow during the process of excitation contraction coupling and on myofibrillar sensitivity (4). However, chronic or overstimulation of the adrenergic system of the heart causes harmful effects on contractile function (7, 11, 24, 28, 33). We have previously reported that, after 10 wk of ovariectomy, there is an upregulation of β\(_1\)-adrenergic receptors in cardiac plasma membrane vesicles compared with sham control, which is completely restored by E\(_2\) supplementation (37) or by exercise training (8). Despite controversial data in the expression of β\(_1\)-adrenoceptors, a sustained and elevated noradrenaline spillover resulting in chronic stimulation of the receptors has been demonstrated in diabetic hearts (15). There is also evidence of sex hormone-related loss of cardiac protec-
tion through reduced expression of HSP72 in OVX hearts (8, 39). This loss of cardioprotective effect was reversed in OVX rats subjected to E2 supplementation (39) or exercise training (8). Similarly, downregulation of HSP72 has been documented in diabetic hearts in which the impaired HSP protection is also offset by endurance exercise (1). Whether deficiency of estrogen and insulin interactively induces a synergistic effect through increased β1- adrenergic stimulation and/or loss of protective effect via reduced HSP72 expression on the cardiac contractile response to Ca^{2+} remains to be elucidated.

The present study was designed to evaluate the influence of diabetes on changes in the response to Ca^{2+} of OVX cardiac myofilaments. We compared the − log free Ca^{2+} concentration (pCa)-myofilament ATPase relationship of isolated myofilamental preparations from sham, ovariectomized, diabetic, diabetic-ovariectomized, and diabetic-ovariectomized rats hearts supplemented with estrogen, insulin, or estrogen plus insulin. We also compared the density and binding affinity of cardiac β1-adrenoceptors in sarcolemmal preparations from these hearts to probe for changes in the effects induced by hormone deficiency. In addition, comparisons of HSP72 content among these hearts were analyzed to probe for changes in this protective factor. Our results showed neither an additive nor synergistic effect of estrogen and insulin on the cardiac contractile response to Ca^{2+}, indicating a similar final common pathway of the hormone action on cardiac contractile activation. Our results also confirm that hypersensitivity of myofilament to Ca^{2+} is specifically induced in ovarian sex hormone-deprived heart even under diabetes complication and that alterations in expression of β1-adrenergic receptors account in part for the underlying cardioprotective effects of estrogen that act together with the hypersensitivity of the myofilaments in determining the gender difference on cardiac activation.

MATERIALS AND METHODS

Animals. Female Sprague-Dawley rats weighing between 180 and 200 g (8–9 wk old) were randomly divided into seven experimental groups, including sham (SHAM), OVX, diabetic (DM), diabetic-ovariectomized (DM-OVX), and diabetic-ovariectomized supplemented with estrogen (DM-OVX + E2), insulin (DM-OVX + INS), or estrogen plus insulin (DM-OVX + E2 + INS) groups. Rats were first sham-operated or ovariectomized as previously described (8). After surgery (2 days), sham rats were subcutaneously injected with 0.1 ml of corn oil, whereas OVX rats were randomly divided into two groups and subcutaneously injected with either 0.1 ml of corn oil with or without 5 μg of 17β-estradiol three times per week for 10 wk. After surgery (2 wk), both sham and OVX rats were further randomly divided into nondiabetic and diabetic groups. Diabetes in rats was induced by intraperitoneal injection of freshly prepared streptozotocin (65 mg/kg body wt), whereas nondiabetic groups were injected with citrate buffer. After diabetic induction (3 days), insulin-supplemented rats were subcutaneously injected with 5 units of human insulin on a daily basis for the whole experimental period. We verified diabetic status by determining urinary glucose using a glucose strip on the day after induction and on the day before the rats were killed. Sufficiency of ovariectomy was verified by a decrease in uterine weight. The animal protocol was approved by the Experimental Animal Committee, Faculty of Science, Mahidol University, in accordance with the guidelines of National Laboratory Animal Centre, Thailand.

Cardiac myofibrillar actomyosin Mg^{2+}-ATPase activity. Cardiac myofibrils were prepared from the left ventricles as described by

Table 1. Body weight, heart weight, uterine weight, and %heart weight/body weight

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body Wt</th>
<th>Heart Wt</th>
<th>Uterine Wt</th>
<th>Heart Wt/Body Wt, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>270±3</td>
<td>0.90±0.01</td>
<td>0.41±0.01</td>
<td>0.33±0.02</td>
</tr>
<tr>
<td>OVX</td>
<td>340±6*</td>
<td>1.00±0.02*</td>
<td>0.09±0.01*</td>
<td>0.29±0.01*</td>
</tr>
<tr>
<td>DM</td>
<td>228±9*†</td>
<td>0.81±0.01†</td>
<td>0.31±0.01†</td>
<td>0.36±0.02†</td>
</tr>
<tr>
<td>DM-OVX</td>
<td>246±9*†</td>
<td>0.92±0.03†</td>
<td>0.09±0.00*</td>
<td>0.37±0.02†</td>
</tr>
<tr>
<td>DM-OVX + E2</td>
<td>233±5*†</td>
<td>0.86±0.02†</td>
<td>0.37±0.03†</td>
<td>0.37±0.02†</td>
</tr>
<tr>
<td>DM-OVX + INS</td>
<td>355±8*</td>
<td>1.01±0.01*</td>
<td>0.09±0.01*</td>
<td>0.29±0.01*</td>
</tr>
<tr>
<td>DM-OVX + E2 + INS</td>
<td>263±4†</td>
<td>0.90±0.01†</td>
<td>0.40±0.01†</td>
<td>0.34±0.01†</td>
</tr>
</tbody>
</table>

Values are means ± SE of 8–10 rats. OVX, ovariectomized; DM, diabetes; DM-OVX, diabetic-ovariectomized; E2, estrogen; INS, insulin. P < 0.05, significant difference from SHAM (*) and OVX (†) groups.
Pagani and Solaro (32). Ca\(^{2+}\)/H\(_{11001}\)-dependent Mg\(^{2+}\)/H\(_{11001}\)-ATPase activity of isolated myofibrils was assayed by determination of inorganic phosphate released in a 10-min linear reaction at 30°C in 2 mM Mg\(^{2+}\)/H\(_{11001}\), 60 mM imidazole, 5 mM MgATP\(^{2-}\), pH 7.0, ionic strength of 120 mM, and various concentrations of Ca\(^{2+}\) ranging from pCa 7.5 to 4.875. Total concentrations of CaCl\(_2\), EGTA, KCl, MgCl\(_2\), and ATP were calculated using a computer program generated from the dissociation constants given by Fabiato (13). The concentration of inorganic phosphate was measured by the method of Carter and Karl (10).

Cardiac membrane preparation. Cardiac membrane was prepared from the left ventricle by the method of Baker and Potter (2) with modifications. In brief, the left ventricle was homogenized in ice-cold 10 mM Tris/H\(_{18528}\)HCl, pH 8.0, and then incubated in 1 M KCl to dissolve the myofilament proteins. Subsequently, the homogenate was filtered through several layers of cheesecloth, and the filtrate was then centrifuged at 43,900 g, 4°C for 20 min. The pellet was resuspended in ice-cold 50 mM HEPES buffer, pH 8.0, in a Teflon glass homogenizer and was immediately used for receptor binding assay after determining the protein content by the Bradford protein assay kit (Bio-Rad).

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1-Adrenergic receptor binding assay. Binding assay for \(\beta_1\)-adrenergic receptor was performed under equilibrium conditions in various concentrations of [\(^{3}\)H]dihydroalprenolol (sp act 92 Ci/mmol; Amersham Pharmacia Biotech) as previously described (37). Nonspecific binding was analyzed in a parallel set of experiment with the addition of (\(-\))alprenolol, a specific antagonist of \(\beta_1\)-adrenergic receptor. The saturation binding was determined from the relationship between specific binding and free ligand using nonlinear least-square regression analysis. Binding parameters, including the density and dissociation constant of the receptors, were determined from a linear transformation of data to the Scatchard plot of bound/free to bound form.

General methods and statistics. The amounts of \(\beta_1\)-adrenergic receptor and HSP72 were determined by Western blot analysis of left ventricular homogenates using polyclonal antibody against \(\beta_1\)-adrenergic receptor and HSP72, respectively.
ergic receptor (Affinity Bioreagents, Golden, CO) and HSP72 (Stressgen, Victoria, British Columbia, Canada) and horseradish peroxidase-labeled secondary antibody, with visualization by ECL (Amersham Pharmacia). MHC isoforms of left ventricular trabeculae were separated electrophoretically as previously described (30). Bands from Western blots were quantified using a GS800 densitometer (Bio-Rad). Data are presented as means \( \pm SE \) from 7–8 preparations. \( #P < 0.05, \) significant difference from DM-OVX group using Student-Newman-Keuls test after ANOVA.

**Materials.** Chemicals were purchased from Sigma Chemical (St. Louis, MO) and Fisher Scientific (Pittsburgh, PA), human insulin was from Eli Lilly (Indianapolis, IN), and glucose strips were from Roche (Indianapolis, IN).

**RESULTS**

Table 1 summarizes body, heart, and uterine weights of all seven experimental groups, namely, sham, OVX, DM, DM-OVX, and DM-OVX supplemented with E2, INS, or E2 + INS. As expected, uterine weights were significantly decreased in OVX and DM-OVX groups compared with sham controls and increased upon E2 supplementation. Uterine weights of DM rats were also significantly lower than shams but in a smaller magnitude compared with ovarian sex hormone-deficient groups. Although significant increases in both heart and body weight were demonstrated in OVX rats, significant decreases in both heart and body weight were detected in DM rats. A decrease in body weight was still observed in the DM-OVX rats without insulin supplementation. Similarly, hypertrophy of the heart represented by an increased heart weight-to-body weight ratio was demonstrated in DM and DM-OVX groups without insulin supplementation.

pCa-myofilament ATPase relationships were compared with evaluate the interactive effect of E2 and INS deprivation on the cardiac myofilament response to Ca\(^{2+}\). As shown in Fig. 1 and summarized in Table 2, maximum myofibrillar ATPase activity of OVX and DM groups was significantly depressed to the same degree (22.7 and 32.9%, respectively) compared with Fig. 4.

Fig. 4. A: pCa-%maximum ATPase activity relation. B: comparison of pCa\(_{50}\) from DM-OVX rats with estrogen and/or insulin supplementation. Data are means \( \pm SE \) from 7–8 preparations. \# \( P < 0.05, \) significant difference from DM-OVX group using Student-Newman-Keuls test after ANOVA.

Fig. 5. Myosin heavy chain (MHC) region of SDS gels and comparison of the relative amount of \( \alpha \)-MHC (as percentage of total MHC) of left ventricular trabeculae from SHAM, OVX, DM, and DM-OVX rats (A) and from DM-OVX hearts with estrogen and/or insulin supplementation (B). Data are means \( \pm SE \) from 8 hearts. \( *P < 0.05, \) significant difference from SHAM (*), OVX (‡), DM-OVX (#), and DM-OVX with insulin supplementation (†) groups, respectively, using Student-Newman-Keuls test after ANOVA.
shams. Maximum myofibrillar ATPase activity was also depressed in DM-OVX rats to a similar degree (29.43%), indicating a lack of additive effect when compared with OVX or DM rats, and ATPase activity was completely restored upon supplementation with both E2 and INS (Fig. 2). On the other hand, the leftward shift in the pCa-myofilament ATPase activity relationship, representing an increase in myofilament sensitivity to Ca$^{2+}$/H$^{1+}$ (reported as pCa50), was only detected in OVX but not in DM rats when compared with sham controls (Fig. 3 and Table 2). The myofilament Ca$^{2+}$/H$^{1+}$ hypersensitivity detected in sex hormone-deficient hearts was also observed in DM-OVX rat hearts (Fig. 3) and reversed upon E2 supplementation (Fig. 4 and Table 2). In all groups, there were no significant differences in the Hill coefficient of the pCa-myofilament ATPase relationship among all groups of animals (Table 2). Analysis of MHC isoforms demonstrated significant reductions in the relative amount of $\alpha$-MHC in both OVX (~28%) and DM (~65%) rat hearts from shams, with a more pronounced suppression in the DM group (Fig. 5A). The same pronounced degree of $\alpha$-MHC suppression detected in DM rats was also observed in the DM-OVX group (Fig. 5A), which was completely restored only by E2 and INS supplementation (Fig. 5B). These results indicate that estrogen and insulin affect the cardiac contractile activation partly through a common final pathway of myosin isoform expression, whereas deficiency of E2 induces an adaptive response of the myofilament to become more sensitive to Ca$^{2+}$ even under diabetes complication.

To determine whether alterations in the $\beta_1$-adrenergic receptor in these hearts were associated with changes in the myofilament response to Ca$^{2+}$, we measured the density, binding affinity, and protein content of $\beta_1$-adrenergic receptor in these hearts using a sarcolemmal preparation and left ventricular homogenate. Similar to the increased Ca$^{2+}$ sensitivity of the myofilament, a significant increase in the $\beta_1$-adrenoceptor density was observed only in OVX (~23%) but not in DM rats compared with shams (Fig. 6A). Moreover, a similar magnitude of enhancement in $\beta_1$-adrenoceptor density was also observed in the DM-OVX group (~17%), which was completely restored upon E2 supplementation (Fig. 6B). In agreement with data of receptor density, results from Western blot analysis using a specific anti-$\beta_1$-adrenergic receptor antibody demonstrated a significant increase in $\beta_1$-adrenergic receptor content in OVX (~48%) and DM-OVX (~49%) groups, and the receptor upregulation in DM-OVX rat hearts could be completely reversed by E2 supplementation (Fig. 7). In all groups, there were no differences in the binding affinity of $\beta_1$-adrenergic receptor among the groups, as summarized in Fig. 8. Thus alterations in $\beta_1$-adrenergic receptor expression underlie...
the protective role of estrogen in the cardiac contractile response to Ca\(^{2+}\).

To further investigate whether changes in the myofilament response to Ca\(^{2+}\) were associated with loss in the cardioprotective effect through expression of HSP72, we determined the amount of this factor using immunoblot analysis. As shown in Fig. 9A, the same magnitude of decrease in HSP72 content was demonstrated in OVX (~27%) and DM (~25%) hearts compared with shams. Similarly, the expression of HSP72 in DM-OVX rats was suppressed to a comparable degree (~22%) compared with shams and increased upon supplementation with both E\(_2\) and INS (Fig. 9B). These results demonstrated that loss in cardioprotective effect through decreased HSP72 expression in sex hormone- or insulin-deficit heart parallels the suppression of maximum myofibrillar ATPase activity but not the hormone-associated hypersensitivity of myofilament to Ca\(^{2+}\).

**DISCUSSION**

The present study is the first to report of a dominant effect of ovariectomy in inducing an enhanced Ca\(^{2+}\) sensitivity of cardiac myofilament under diabetes complication, emphasizing the significance of enhanced myofilament Ca\(^{2+}\) sensitivity in the pathophysiology of the heart in ovariectomy. We also observed the mechanistic adaptation underlying the cardioprotective effects of estrogen that involves elevated expression of β\(_1\)-adrenergic receptor and loss of protective factor, HSP72. Moreover, estrogen and insulin physiologically regulate and protect cardiac contractile function by an interactive action of the hormones on the maximum myofibrillar ATPase activity but not the myofilament Ca\(^{2+}\) sensitivity.

In ovariectomy, the close relation between enhanced myofilament sensitivity to Ca\(^{2+}\) and increased β\(_1\)-adrenoceptor expression in the heart, with or without complication of diabetes, confirms the adaptation of the contractile response of the heart in a pathological direction. Previous demonstrations that in ovariectomy there is a decrease in intracellular cardiac Ca\(^{2+}\) concentration (34) and sarcoplasmic reticulum Ca\(^{2+}\) uptake activity (9) suggest that both myofilament Ca\(^{2+}\) hypersensitivity and upregulation of β\(_1\)-adrenoceptors are likely maladaptive responses induced after sex hormone depletion. The increased adrenergic drive either through upregulation of β\(_1\)-adrenoceptors or increase in signaling process is known to be toxic to the heart (7, 11, 24, 28, 33). In a transgenic mouse model, overexpression of human β\(_1\)-adrenergic receptors in the heart produces a short-lived improvement of cardiac function..
but ultimately leads a cardiomyopathic phenotype characterized by dilatation and depressed contractile functions (5, 12). This harmful compensatory mechanism of the heart induced by chronic adrenergic stimulation has provided the fundamental basis for the use of anti-adrenergic agents in treatment of chronic heart failure (6, 21, 27, 31). Although the sequential induction between changes in the myofilament response to Ca\(^{2+}\) and in \(\beta_1\)-adrenoceptors in ovariectomy remains unclear, parallel changes in these factors even with diabetes complication provide evidence for a high potential of cardiomyopathy induction in sex hormone-deficient hearts. Moreover, although interactive effects of E\(_2\) and insulin on the ovariectomy-associated increase in the myofilament response to Ca\(^{2+}\) are absent, E\(_2\) demonstrates a cardioprotective effect over insulin in preventing Ca\(^{2+}\) hypersensitivity of myofilaments. This absence of hormone interaction confirms that Ca\(^{2+}\) hypersensitivity of myofilaments is a specific maladaptive response of the heart induced by E\(_2\) deficiency. Physiological suppression of \(\beta_1\)-adrenoceptor expression and stimulation may in part account for the cardioprotective effect of E\(_2\) on the cardiac contractile response to Ca\(^{2+}\).

It is not clear how the increase in myofilament response to Ca\(^{2+}\) seen in our study leads to cardiac contractile dysfunction in ovariectomy. Enhanced Ca\(^{2+}\) sensitivity of the myofilament is a common feature in most cardiomyopathy patients (16) and heart failure models (20, 42, 43). Increased myofilament response to Ca\(^{2+}\) is the cellular mechanism proposed for alterations in the Starling force of the heart (35) and could provide a therapeutic approach in the search for Ca\(^{2+}\)-sensitizing agents for the heart (23). Elevated Ca\(^{2+}\) regulation of cardiac muscle activation has been shown to be the primary mechanism contributing to pathogenesis of troponin T-linked familial hypertrophic cardiomyopathy (19). Increased affinity of Ca\(^{2+}\) bound to myofilament occurring with mutant cardiac troponin I could also cause a threat for arrhythmic activity associated with cardiomyopathy (26). Moreover, a chronic increase in the cardiac myofilament response to Ca\(^{2+}\) could cause hypertrophic induction in association with mutations in sarcomeric proteins (16). Besides the reported shift in cardiac MHC isoforms in ovariectomy (8, 41) that is more likely to underlie the suppressed maximum myofibrillar ATPase activity, evidence for changes in other sarcomeric proteins that subsequently alter the myofilament response to Ca\(^{2+}\) awaits future studies.

Differential effects of E\(_2\) and insulin interaction on cardiac contractile function despite the presence of both receptors in the myocardium (17, 38) suggest that different mechanisms exist for the hormones on the cardiac contractile response to Ca\(^{2+}\). In contrast to other organs, the reversal of maximum cardiac myofibrillar ATPase activity and \(\alpha\)-MHC expression in ovariectomy combined with diabetes only results when both E\(_2\) and insulin treatment are given, reflecting an interaction of the hormones in activating myofilament function. On the other hand, the absence of interaction of the hormones on the ovariectomy-associated increase in cardiac myofilament Ca\(^{2+}\) sensitivity confirms that Ca\(^{2+}\) hypersensitivity of myofilament is a specific maladaptive response of the heart induced by sex hormone deficiency. How the hormones act on cardiac contractile function is not known at present.

Inasmuch as the stability and quality control of protein folding after translation in cardiomyocytes are accounted for by the action of a biological chaperone, HSP72 (3), parallel changes in HSP72 level and maximum myofibrillar ATPase activity (Figs. 1, 2, and 9) provide a potential common target for E\(_2\)- and insulin-controlling process, namely via HSP72 function. There are reports showing that both E\(_2\) and insulin control HSP72 expression via phosphorylation of the same transcription heat shock factor-1 (1, 25, 44).

Although homeostatic balance of \(\beta_1\)-adrenoceptor receptor and protective factor HSP72 is physiologically regulated by E\(_2\), it is likely that only protective factors are regulated by insulin. Our data confirm the physiologically cardioprotective function of E\(_2\) on the contractile response to Ca\(^{2+}\) even under diabetes complication. These results provide further support for the beneficial use of E\(_2\) and \(\beta_1\)-blocker in preventing maladaptation of the heart to estrogen deficiency, thereby lowering the incidence of heart failure in postmenopausal women.

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