
METHODS

Studies were performed with 8-wk-old female Sprague-Dawley (Harlan Hsd-LSD) rats weighing ~200 g at surgery. Rats were housed under standard environmental conditions and maintained on rat chow (see Dietary data for details) and tap water ad libitum. All experimental procedures were approved by our Institution’s Animal Care and Use Committee. Anesthesia for surgical procedures was induced with xylazine (7 mg/kg) and ketamine (62 mg/kg) administered by intraperitoneal injection. Postoperative analgesia was provided by buprenorphine HCl (0.025 mg/kg sc) administered to the rats at the time of surgery. At the experimental endpoint, animals were anesthetized with pentobarbital sodium (50 mg/kg ip). A total of eight groups of age-matched female rats were studied as follows: sham-operated controls fed a phytoestrogen-free or high-phytoestrogen diet (sham and sham-phyto rats, respectively), intact rats with fistula fed a phytoestrogen-free or high-phytoestrogen diet (Fist and Fist-phyto rats, respectively), ovariectomized rats with fistula fed a phytoestrogen-free or high-phytoestrogen diet (Fist-ox and Fist-ox-phyto rats, respectively), and ovariectomized rats with fistula treated with estrogen and fed a phytoestrogen-free or high-phytoestrogen diet (Fist-ox-EST and Fist-ox-EST-phyto rats, respectively).

Dietary data. Animals were fed either a casein-based phytoestrogen-free rat chow (Purina Mills test diet 5K96) with <1.0 ppm total isoflavones or a high-phytoestrogen diet (Purina Mills RMH 3000) with a total isoflavone content of 417 ± 76 ppm (aglycone units), 196 ± 33 ppm genistin, and 183 ± 7 ppm daidzein. The crude protein content of these diets is similar (~19–22%). Rats were fed their respective diets for 1 wk before the fistula surgery and throughout the 8-wk experimental protocol.

Surgical procedures. Infrarenal AV fistula was created in rats as previously described (9). Briefly, a ventral abdominal laparotomy was performed to expose the aorta and caudal vena cava. An 18-gauge needle was inserted into the exposed abdominal aorta below the renal arteries and advanced into the vena cava to create the fistula. The needle was withdrawn, and the aortic puncture site was sealed with
cyanoacrylate. Abdominal musculature and skin incisions were closed by standard techniques with absorbable suture and autoclips, respectively. For the ovariectomy study groups, the ovaries were isolated, the ovarian pedicles were ligated, and the ovaries were excised. Ovariectomies were performed 5 days before the fistula surgery. Estrogen was administered via subcutaneous time-release pellets (0.25 mg total, 60-day release; Innovative Research of America, Sarasota, FL), which delivered a dose of ~0.02 mg·kg⁻¹·day⁻¹ and were implanted at the time of ovariectomy (13). Plasma samples from each animal were assayed for estrogen content using a commercial radioimmunoassay (Coat-a-Count kit; DPC, Los Angeles, CA).

Experimental protocol. All rats were studied at 8 wk after creation of the AV fistula. This time period was chosen because, in previous studies with male rats, a significant number of rats have been shown to develop symptomatic congestive heart failure (CHF) by 8 wk postfistula (9, 16). Results from the sham, Fist, and Fist + phyto groups have been previously published and are included herein for comparison purposes (9, 17). At the experimental endpoint, each rat was weighed and anesthetized; after laparotomy, fistula patency was visually confirmed. Rats were then attached to a ventilator, and, after thoracotomy to expose the heart, cardiac output was measured using a Doppler flow probe (model 3SB; Transonic Systems, Ithaca, NY). A perfusion cannula was then introduced into the aorta, and the heart was removed and attached to a perfusion apparatus (see Assessment of ventricular size and function below). After completion of the functional studies, the atria and great vessels were removed, and the LV (including septum) and right ventricle (RV) were separated and weighed. Lung and uterine weights, after removal of the ovaries, were also measured.

Assessment of ventricular size and function. LV volume and function were evaluated with a blood-perfused isolated heart preparation as previously described (9, 16, 17). Briefly, arterial blood from the carotid artery of a support rat was pumped to a pressurized reservoir for retrograde perfusion of the experimental heart. Coronary perfusion pressure was maintained at 100 ± 5 mmHg. The coronary venous effluent was collected and returned to the support rat through a jugular vein catheter. Before excirpitation of the heart from the anesthetized rat, the coronoid arteries were ligated, and a cannula was inserted into the thoracic aorta and secured with a silk ligature. The heart was then quickly removed from the chest and attached to the apparatus. LV developed pressure was continuously recorded with a pressure transducer attached to a latex balloon inserted through the mitral valve orifice into the LV chamber. Once the heart developed stable isovolumetric contractions, the balloon volume that produced an LV end-diastolic pressure-volume relation (LVEDPR) of 0 mmHg (V0) was determined. V0 was then increased in 5- to 10-μl increments from this point until an LVEDV of 25 mmHg was attained. The balloon was then evacuated, and this procedure was repeated until a minimum of three consistent runs were obtained.

Western blot analysis of estrogen receptor expression. Total proteins were extracted from LV tissue and homogenized with protease inhibitor cocktail (Pierce). Protein concentrations were determined (Bio-Rad), and equal samples were then separated by 10% SDS-PAGE and transferred onto nitrocellulose membranes (Amersham). Membranes were blocked with 5% nonfat milk in TBS and 0.01% Tween 20 for 1 h at room temperature and then incubated with rabbit anti-estrogen receptor (ER)-α polyclonal antibody (MC-20; Santa Cruz). After incubation with primary antibody, the membranes were rinsed in TBS containing 0.01% Tween 20 and incubated for 1 h in the 5% milk buffer with peroxidase-coupled donkey anti-rabbit IgG (Santa Cruz). Immunoreaction signals were visualized with enhanced chemiluminescence (Pierce) by exposure to hyperfilm (RPI). Ponceau-S staining was used to confirm equal loading and for normalization of ER-α signal. Densitometry analysis was performed with a Kodak molecular image station 2000MM. Although several commercial antibodies were tried, none provided the specificity needed to allow repeatable measurement of LV ER-β expression.

Data and statistical analysis. The end-diastolic and peak isovolumetric pressures were determined after each increase in V0 (AD-Instruments chart version 5, Colorado Springs, CO). Further processing and statistical analyses were performed with GraphPad analysis software (GraphPad 4.0; Prism, San Diego, CA). LV volumes were adjusted to account for the volume displaced by the balloon material. Pressure-volume relations generated for the balloon alone indicated that the balloon’s contribution to LVEDP was negligible over the experimental LV volume range. Diastolic LV pressure-volume data were fitted with a third-order polynomial regression, and V0 for each pressure-volume curve was calculated. With the use of this regression equation, LV end-diastolic volumes (LVEDVs) were calculated at discrete LVEDP intervals (each 2.5 mmHg) for graphical comparison. Systolic LV pressure-volume data were fitted with linear regression, and the slopes of these relations were used as indexes of intrinsic chamber contractility (i.e., neurohormonal influence removed). Linear relations were statistically compared by analysis of covariance. Grouped data comparisons were made by one-way ANOVA, and comparisons of discrete data points on nonlinear relations were analyzed by two-way ANOVA. When a significant F-ratio (P < 0.05) was obtained, intergroup comparisons were made with a modified t-test and Bonferroni bounds. Statistical significance was taken to be P < 0.05/k, where k is equal to the number of groups compared (35). To evaluate the interaction of the estrogen and phytoestrogen treatments, a two-way ANOVA was performed on the ovariectomized groups.

RESULTS

Group-averaged cardiac output and LV, RV, lung, uterine, and body weights for the combined sham and 8-wk Fist groups are listed in Table 1. Because there were no significant differences in parameters between the two sham-operated groups (sham and sham + phyto), these groups were combined for presentation. Cardiac output was significantly increased four-fold in the fistula animals, confirming the successful creation of fistula and chronic volume overload conditions. LV and RV weights were significantly increased for all of the Fist

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight, g</th>
<th>LV Weight, mg</th>
<th>RV Weight, mg</th>
<th>Lung Weight, mg</th>
<th>Uterine Weight, mg</th>
<th>Cardiac Output, ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham (n = 17)</td>
<td>237 ± 16†</td>
<td>620 ± 26†</td>
<td>159 ± 27†</td>
<td>1,305 ± 96†</td>
<td>701 ± 177†</td>
<td>27 ± 7†</td>
</tr>
<tr>
<td>Fist (n = 11)</td>
<td>259 ± 11†</td>
<td>1,184 ± 229*</td>
<td>372 ± 92*</td>
<td>1,725 ± 423†</td>
<td>615 ± 162†</td>
<td>128 ± 24*</td>
</tr>
<tr>
<td>Fist-OX (n = 12)</td>
<td>330 ± 23*</td>
<td>1,319 ± 154*</td>
<td>436 ± 84*</td>
<td>2,192 ± 448*</td>
<td>96 ± 23*</td>
<td>151 ± 29*</td>
</tr>
<tr>
<td>Fist-OX + EST (n = 11)</td>
<td>223 ± 12†</td>
<td>1,059 ± 142†</td>
<td>320 ± 54†</td>
<td>1,465 ± 412†</td>
<td>491 ± 135†</td>
<td>118 ± 24†</td>
</tr>
<tr>
<td>Fist + phyto (n = 8)</td>
<td>241 ± 16†</td>
<td>1,079 ± 199†</td>
<td>335 ± 88*</td>
<td>1,559 ± 192†</td>
<td>730 ± 180†</td>
<td>114 ± 15*</td>
</tr>
<tr>
<td>Fist-OX + phyto (n = 8)</td>
<td>357 ± 24*</td>
<td>1,381 ± 121*</td>
<td>504 ± 94*</td>
<td>2,254 ± 464*</td>
<td>108 ± 18*</td>
<td>166 ± 40*</td>
</tr>
<tr>
<td>Fist-OX + EST + phyto (n = 11)</td>
<td>221 ± 18†</td>
<td>1,128 ± 184*</td>
<td>340 ± 69†</td>
<td>1,478 ± 257†</td>
<td>477 ± 96†</td>
<td>122 ± 41*</td>
</tr>
</tbody>
</table>

Values are means ± SD. LV, left ventricle; RV, right ventricle; phyto, high-phytoestrogen diet; Sham, sham-operated control; Fist, fistula; OX, ovariectomized; EST, estrogen. *P < 0.05 vs. Sham. †P < 0.05 vs. Fist-OX.
groups relative to sham controls, consistent with our previous results (16). Also, collagen volume fraction, as measured by picrosirius red staining of fixed LV midventricular sections, was not significantly different between groups (data not shown). Successful excision of ovarian tissue was confirmed by a substantial 86% decrease (\( P < 0.05 \)) in uterine weights in the ovariectomized rats, as well as a significant 67% reduction in serum estrogen levels relative to results in sham rats (Fig. 1). Estrogen replacement sustained uterine weights in ovariectomized rats (31% reduction vs. Sham) and resulted in serum estrogen levels comparable to those for the intact groups. The phytoestrogen content of the diet had no effect on uterine weights or serum estrogen levels.

LV ER-\( \alpha \)-expression was significantly reduced in the Fist-OX group (\( P < 0.05 \) vs. Sham) and maintained in rats treated with estrogen (Fig. 2). The intact rats with fistula fed a high-phytoestrogen diet had greater LV ER-\( \alpha \) expression than their counterparts fed a phytoestrogen-free diet (\( P < 0.01 \)), whereas expression in sham animals was not affected by diet (data not shown). Although this dietary phytoestrogen-induced increase in ER-\( \alpha \) expression was also evident in the ovariectomized rats and ovariectomized rats treated with estrogen, these differences did not reach significance.

Hormonal effects on cardiac remodeling and function. The Fist-OX group developed significant hypertrophy relative to the sham group (113%); however, this increase was not different from the intact Fist group. Estrogen replacement in the ovariectomized rats attenuated this LV hypertrophy (20% less than that of Fist-OX; \( P < 0.05 \)). Estrogen treatment also significantly attenuated the RV hypertrophy in the Fist-OX+EST animals (28% less than that of Fist-OX). Lung weights in the Fist and Fist-OX rats were significantly increased relative to sham controls, with the greatest increase (68%) occurring in the Fist-OX group. Estrogen replacement prevented this increase in lung weight in the ovariectomized rats postfistula (Fist-OX+EST vs. sham; \( P = \) not significant). Rats were classified to have CHF if they developed a rapid increase in body weight (>50 g/wk) and displayed lethargy and labored respiration; this condition was confirmed at the experimental endpoint by lung weights exceeding 2 g and the presence of ascites. With the use of these criteria, 27% of the intact Fist animals and 75% of Fist-OX group developed CHF. In contrast, only one of the Fist-OX+EST animals developed CHF.

Group differences in the average LVEDP-LVEDV relationship are depicted in Fig. 3A and summarized in Table 2. Structural dilatation of the LV resulting from enlarged chamber dimensions was assessed by the change in unstressed LV volume (\( V_0 \)). In the absence of altered compliance, this remodeling would characteristically result in a parallel rightward shift of the LVEDP-LVEDV relationship. Both intact and ovariectomized females developed significant structural dilatation of the LV postfistula, with the ovariectomized group developing a substantially greater degree of LV chamber dilatation (43% and 67% increases relative to sham control, respectively). Although this LV dilatation was significantly attenuated by estrogen treatment (23% decrease relative to Fist-OX), the Fist-OX+EST rats still developed LV dilatation that was comparable to that of the intact females (28% increase, Fist-OX+EST vs. Sham; \( P < 0.05 \)). In addition to structural dilatation,
dilatation, changes in compliance, as measured by the volume required to increase LVEDP from 0 to 25 mmHg, occurred as a result of sustained volume overload. Increased compliance is represented as a nonparallel rightward shift of the LVEDP-LVEDV relation. Hearts from Fist and Fist-OX rats exhibited significant increases in ventricular chamber compliance, but the absence of ovarian hormones exacerbated this increase in compliance nearly fourfold (134% and 426% relative to Sham, respectively). Estrogen replacement significantly attenuated this development of increased chamber compliance in the Fist-OX group (203% relative to Sham; *P < 0.05 vs. Fist, †P > 0.05 vs. Fist-OX).

Figure 4A depicts the influence of ovarian hormones on the average LV M/V postfistula. The intact Fist animals developed a significant increase in LV M/V over the full range of LVEDP compared with the sham group, whereas the Fist-OX group did not. However, the Fist-OX + EST group developed significantly increased LV M/V, which was comparable to that of the intact Fist group. The effects of volume overload-induced ventricular remodeling on systolic function were assessed with the linear systolic LV pressure-volume relations (slopes reported in Table 2), previously validated by Suga et al. (43) as an accurate index of LV chamber contractility. Chamber contractility was markedly reduced in hearts from intact females postfistula relative to sham-operated controls (39% reduction vs. Sham; *P < 0.05). However, ovariectomized females postfistula had a much greater reduction in the slope of the systolic pressure-volume relation (77% reduction vs. Sham; *P < 0.05), which was only partially attenuated by estrogen replacement (64% reduction relative to Sham; *P < 0.05).

Dietary effects on cardiac remodeling and function. As can be seen in Table 1, the high-phytoestrogen diet did not appreciably affect the extent of LV or RV hypertrophy in the Fist/phyto or Fist-OX/phyto groups (74% and 123% increase, respectively). However, the absence of ovarian hormones exacerbated this increase in cardiovascular mass nearly fourfold (134% and 426% relative to Sham, respectively). Estrogen replacement significantly attenuated this development of increased vascular mass in the Fist-OX group (203% relative to Sham; *P < 0.05 vs. Fist, †P > 0.05 vs. Fist-OX).

Table 2. Diastolic and systolic cardiac functional parameters from isolated hearts

<table>
<thead>
<tr>
<th>Group</th>
<th>Vo, µl</th>
<th>ΔVo–25/25, µl/mmHg</th>
<th>Slope of Systolic Pressure-Volume Relation, mmHg/µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham (n = 11)</td>
<td>300±14†</td>
<td>2.26±0.30†</td>
<td>1.10±0.10†</td>
</tr>
<tr>
<td>Fist (n = 6)</td>
<td>428±16†</td>
<td>5.29±0.80†</td>
<td>0.67±0.08†</td>
</tr>
<tr>
<td>Fist-OX (n = 5)</td>
<td>501±11*</td>
<td>11.89±0.97*</td>
<td>0.25±0.03*</td>
</tr>
<tr>
<td>Fist-OX + EST (n = 9)</td>
<td>384±24†</td>
<td>6.84±1.10†</td>
<td>0.40±0.05†</td>
</tr>
<tr>
<td>Fist+phyto (n = 7)</td>
<td>286±17†</td>
<td>2.80±0.44†</td>
<td>1.18±0.13†</td>
</tr>
<tr>
<td>Fist-OX+phyto (n = 8)</td>
<td>494±15*</td>
<td>11.66±0.99*</td>
<td>0.24±0.04*</td>
</tr>
<tr>
<td>Fist-OX+EST+phyto (n = 10)</td>
<td>295±19†</td>
<td>4.67±1.30†</td>
<td>0.66±0.10†</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 vs. Sham. †P < 0.05 vs. Fist-OX. ‡P < 0.05 vs. Fist-OX + EST. Vo, LV volume at an end-diastolic pressure of 0 mmHg; ΔVo–25, change in LV volume between end-diastolic pressure of 0 and 25 mmHg.
crease in LV mass, respectively, vs. Sham; \( P < 0.05 \). Although the phytoestrogen diet did not prevent the significant increases in lung weights developed by the Fist-OX+phyto rats, lung weights in the intact Fist+phyto group were not significantly different from those of control. Furthermore, although 75% of the Fist-OX+phyto rats developed CHF, none of the Fist+phyto group and only one of the Fist-OX+EST+phyto rats developed CHF.

A plot of the LVEDP-LVEDV relationship in Fig. 3B depicts the effects of dietary phytoestrogens on the group-averaged diastolic function of isolated hearts (parameters summarized in Table 2). Although the high-phytoestrogen diet did not prevent the chamber dilatation and increased compliance developed by the ovariectomized females (i.e., Fist-OX+phyto; \( P = \) not significant vs. Fist-OX), the diet did prevent both the dilatation and increased compliance in the intact Fist+phyto group. This is reflected by the LVEDP-LVEDV relation of the Fist+phyto group being identical to that of the sham group. Likewise, dilatation was prevented and increased compliance was markedly attenuated (\( P < 0.05 \) vs. Fist-OX) in the Fist-OX+EST+phyto group, indicating that estrogen and phytoestrogens act synergistically to prevent volume overload-induced adverse ventricular remodeling. Phytoestrogen diet did not alter the LV M/V in the Fist-OX+phyto group from that seen in the Fist-OX group (Fig. 4B). However, the intact females with fistula fed a high-phytoestrogen diet developed a marked increase in LV M/V relative to the intact females fed a phytoestrogen-free diet (\( P < 0.05 \)). Much like the Fist+phyto group, the combined treatments of phytoestrogen and estrogen produced a comparable increase in LV M/V for the Fist-OX+EST+phyto group (\( P < 0.05 \) vs. Fist-OX, Fist, and sham groups).

LV chamber contractility was maintained in hearts from intact Fist+phyto rats (\( P = \) not significant vs. Sham; Table 2). Although this phytoestrogen-derived cardioprotection was not evident in the Fist-OX+phyto females (78% reduction in contractility vs. Sham), the combined treatments of high-phytoestrogen diet and estrogen replacement did significantly improve the intrinsic contractility of the Fist-OX+EST+phyto group vs. estrogen treatment alone (40 vs. 64% reduction relative to Sham). However, contractility remained significantly impaired in both of the estrogen-treated groups compared with the sham and Fist+phyto hearts. Statistical analysis of the LV dilatation (i.e., \( V_0 \)) and chamber contractility of the four ovariectomized groups determined that the interaction of the phytoestrogen and estrogen treatments was significant.

**DISCUSSION**

Although premenopausal women are essentially protected from cardiovascular disease, the development of CHF in postmenopausal women is exacerbated, with a greater incidence than that shown in age-matched men (18, 21, 24). Historically, this sex-specific cardioprotection has been attributed to estrogen (34); however, this assumption has been refuted by results from clinical trials, including the Heart and Estrogen/Progestin Replacement Study, which reported no cardiovascular benefit of hormone replacement therapy (3, 19). The typical Western diet contains minimal soy phytoestrogens (14, 15, 46), but the emergent use of dietary supplements based on the supposition that they are beneficial is of concern because little is known about how these compounds affect the heart’s ability to respond to stress (11). Although we have previously demonstrated that dietary phytoestrogens provide cardioprotection to intact female rats and contribute to sex differences in ventricular remodeling induced by volume overload, these studies did not address the influence of estrogen or the possibility of interactions between phytoestrogens and estrogen (16, 17). Thus the purpose of the present study was to test the hypothesis that dietary phytoestrogens, in conjunction with estrogen, prevent adverse myocardial remodeling induced by chronic volume overload.

**Hormonal effects.** The compensatory processes and ensuing pattern of myocardial remodeling occurring in the AV fistula model of chronic volume overload mimics those described in humans with CHF (23) and, in male rats, result in the development of myocardial remodeling characterized by eccentric hypertrophy, marked dilatation, increased ventricular compliance, and CHF-related mortality (9). In contrast, intact female rats fed a diet with high-phytoestrogen content develop concentric hypertrophy with no significant ventricular dilatation or alterations in myocardial compliance and minimal mortality (16, 17). These studies, as well as the findings herein, are consistent with the report by Liu et al. (30) in which intact female rats developed significant hypertrophy without progression to heart failure postfistula (unspecified diet). The extent of LV dilatation in ovariectomized females at 8 wk postfistula was comparable to that previously reported in age-matched male rats (i.e., \( V_0 \) of 497 vs. 472 \( \mu \)L, respectively) (16). The marked ventricular dilatation and pulmonary edema exhibited by the ovariectomized females vs. intact females clearly demonstrate the influence of circulating ovarian hormones on the remodeling of the myocardium postfistula. Estrogen replacement significantly attenuated, but did not prevent, the LV hypertrophy, dilatation, and increased compliance occurring postfistula in the ovariectomized rats. However, it is worth noting that the increase in LV mass is not thought to be pathological but rather represents a necessary adaptation that allows for the normalization of increased wall stress imposed by volume overload. Previous findings suggest that failure to adequately increase LV M/V in response to chronic volume overload is indicative of a maladaptive pattern of remodeling that will eventually result in heart failure, as occurs in male rats (16, 23). Figure 4A illustrates that, although intact females successfully increased their LV M/V postfistula relative to sham rats, the ovariectomized females were not able to replicate this response. Because of this inability to normalize the increased wall stress imposed by the volume overload, 75% of the Fist-OX rats developed extensive pulmonary edema, signifying CHF. Estrogen replacement in the Fist-OX group produced a more successful ventricular adaptation in these rats, comparable to that seen in the intact Fist group fed a phytoestrogen-free diet. This positive effect of estrogen treatment on LV remodeling in ovariectomized female rats was similar to that demonstrated by Xu et al. (48).

Although the depressed systolic function in ovariectomized rats was significantly improved by estrogen treatment, it was not maintained at a level comparable to that of intact females. However, despite the relative decrease in systolic function, only one rat in this estrogen-treated group actually developed heart failure. This finding is consistent with that of Beer et al. (6) who found that estrogen treatment of female rats prevented...
the development of heart failure induced by myocardial infarction. Likewise, estrogen replacement led to improved myocardocyte survival in mice after myocardial infarction, and this cardioprotective effect was mediated via activation of the phosphatidylinositol 3-kinase/Akt pathway (39). Sex-specific differences in myocardial Akt activation have been demonstrated and represent a plausible mechanism contributing to cardioprotection in premenopausal women (2, 10). Additionally, our results suggest that the maintenance of estrogen receptor expression in the heart may be key to cardioprotection because the groups with the lowest levels of ER-α expression developed a greater degree of LV dilatation and increased compliance and CHF.

Dietary effects. Although many laboratories, including our own, have demonstrated the cardioprotective effects of dietary soy compounds, the most striking finding in the present study was the synergism of the combined phytoestrogen and estrogen treatments in preventing adverse remodeling (17, 42, 45, 50). The LV dilatation was completely prevented in both the Fist+phyto and Fist-OX+EST+phyto groups, allowing for the development of an appropriate hypertrophic response (i.e., increased LV M/V). Intact females fed the high phytoestrogen diet developed the greatest increase in LV M/V, and the successful compensatory adaptation of this treatment group is reflected in their ability to sustain both diastolic and systolic performance, as well as the complete absence of symptomatic CHF. These cardioprotected females also had the greatest level of LV ER-α expression. Contributing to this successful compensation was the prevention of increased ventricular compliance in the Fist+phyto and Fist-OX+EST+phyto groups. In contrast, the inability of ovariectomized females, regardless of diet, to develop appropriate hypertrophy predisposed them to more extensive ventricular dilatation and increased compliance. These findings clearly demonstrate a synergistic cardioprotective effect of the combined estrogen and phytoestrogen treatments contributing to the successful adaptation of these animals to chronic volume overload. Although the cardioprotective effects imparted on LV chamber structure and compliance were striking, the combined treatment of estrogen and phytoestrogens in ovariectomized rats did not fully maintain systolic cardiac function postfistula, as demonstrated in the intact females that were treated with phytoestrogens. This finding suggests that other ovarian-derived factors, in addition to estrogen, work in conjunction with the phytoestrogenic compounds to further enhance cardioprotection.

Although there are multiple phytoestrogenic compounds derived from soybeans, a likely candidate mediating these dietary phytoestrogenic effects is genistein. Genistein has been shown to interact with estrogen receptors, preferentially binding to ER-β (26). ER-α is the predominant isoform expressed in the heart, but ER-β has been implicated as the cardioprotective subtype, on the basis of studies that utilized ER-α and ER-β knockout mice [reviewed by Harris (20) and Otsuki et al. (37)]. Genistein has been shown to exert both anti-inflammatory and antioxidant effects (29, 33) and can inhibit multiple cellular signaling proteins including MAPK (1, 7, 44), NF-κB (7), and IL-6 (38). These anti-inflammatory effects are potentially important because observations in our laboratory and others indicate that proinflammatory cytokines are centrally involved in the initial response of the heart to insult (4, 23). It should also be noted that genistein has been shown to inhibit the transcription and activation of matrix metalloproteinases (MMPs) in cancer cells (25, 28, 49). Genistein-mediated MMP inhibition represents a plausible cardioprotective mechanism, as our group (12) has previously demonstrated that MMP inhibition markedly attenuates the adverse cardiac remodeling occurring postfistula.

Summary. The synergistic effects of dietary-derived phytoestrogens and estrogen on remodeling in the ovariectomized female rats were marked and led to complete prevention of the LV dilatation and significant attenuation of the increased LV compliance associated with chronic volume overload. These remarkable and unexpected findings provide a basis for future investigations into the mechanisms underlying the complementary synergistic cardioprotective effects of hormonal and phytoestrogenic compounds. Future studies are warranted to determine (1) the specific phytoestrogen(s) responsible, (2) whether these effects are estrogen receptor mediated, (3) and which key signaling pathways are involved. Until the mechanisms responsible for the loss of premenopausal cardioprotection are resolved, the most effective clinical therapy will remain controversial.

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REFERENCES