Effects of ovarian sex hormones and downhill running on fiber-type-specific HSP70 expression in rat soleus

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Bombardier E, Vigna C, Iqbal S, Tiidus PM, Tupling AR. Effects of ovarian sex hormones and downhill running on fiber-type-specific HSP70 expression in rat soleus. J Appl Physiol 106: 2009–2015, 2009.—This study examined the influence of the ovarian sex hormones, estrogen and progesterone, on the fiber-type-specific response of the inducible 70-kDa heat shock protein (HSP70) to damaging exercise in rat soleus. Ovariectomized female rats were divided into three treatment groups (n = 16 per group): sham (S), progesterone (P; 25 mg pellet), and estrogen (E; 0.25 mg pellet). Each treatment group was divided into control and exercised groups. After 8 days of sham or hormone treatment, animals ran downhill intermittently for 90 min (17 m/min, −13.5° grade) on a treadmill, and soleus muscles were removed 24 h postexercise. HSP70 expression was assessed in whole muscle homogenates by Western blotting and in individual muscle fiber types by immunohistochemical analysis of serial cross sections of soleus samples. Comparisons between control groups showed that HSP70 expression in soleus was increased (P < 0.05) in E compared with both S and P. No difference (P > 0.05) was observed between S and P. Following downhill running, HSP70 content in soleus was increased (P < 0.05) compared with control in S and P, but not (P > 0.05) in E. As a result, soleus HSP70 content following downhill running was not different (P > 0.05) between any of the treatment groups. Under all conditions, HSP70 content was higher in type I vs. type II fibers, and the effects of both estrogen and exercise on HSP70 expression in soleus were also more pronounced in type I vs. type II fibers. These results demonstrate that 1) estrogen regulates HSP70 expression in skeletal muscle, increasing basal HSP70 expression and preventing further increases in HSP70 in response to exercise; 2) progesterone is not involved in the regulation of HSP70 expression in skeletal muscle; and 3) the effects of estrogen and exercise on HSP70 expression in skeletal muscle are fiber type specific.

estrogen; progesterone; muscle fiber type

HEAT SHOCK PROTEINS (HSP) are families of highly conserved stress proteins found in almost all cells, which have been shown to be induced by a variety of environmental and intracellular stresses (18). The inducible 70-kDa HSP (HSP70) may be the most highly induced protein of the cellular stress response and is rapidly upregulated under conditions of oxidative stress (48). It is well established that HSP70 content in skeletal muscle increases in response to both nondamaging and damaging exercise and can protect against contraction-induced muscle damage (for review, see Refs. 10, 19). However, the HSP70 response to exercise in both skeletal (31, 33) and cardiac muscle (32) is sex specific, with males demonstrating a more robust response than females. Collectively, these studies and others have shown that the sex-specific HSP70 response to exercise is mediated by the ovarian sex hormone estrogen (for review, see Ref. 29).

Although estrogen inhibits both the cardiac and skeletal muscle HSP70 response to exercise, suggesting that HSP70 expression in muscle is negatively regulated by estrogen, it has been shown that HSP70 content in hearts of ovariectomized female rats is lower compared with age-matched females, and that estrogen treatment increased cardiac HSP70 levels in the ovariectomized rats (47). These findings suggest that estrogen stimulates constitutive or basal HSP70 expression, at least in the heart. Consistent with this view, estrogen treatment has been shown to activate the transcription factor nuclear factor-κB rapidly through a nongenomic mechanism, which activates heat shock factor-1 (HSF-1) in both male and female cardiac myocytes, which results in the induction of HSP70 protein within 10–24 h (38). Given that increased basal HSP70 expression would be expected to be protective in heart (21, 34, 35), and that estrogen itself can act as a membrane stabilizer and antioxidant (44, 50), it stands to reason that the sex differences in cardiac HSP70 response to exercise reflect the fact that female hearts are naturally more protected than male hearts. The independent effects of estrogen on basal HSP70 expression in skeletal muscle have not been described.

A large proportion of the studies examining sex differences have focused predominantly on estrogen; however, Knowlton and Sun (11) found that progesterone independently activated HSF-1 and, consequently, increased the expression of HSP70 in adult male isolated cardiac myocytes. Progesterone has also been demonstrated to have a role in the inflammatory response (4, 9) similar to that of estrogen (42, 43), potentially influencing the HSP70 response to a given stress. It is presently unknown if progesterone regulates basal HSP70 expression in skeletal muscle or whether it influences the muscle HSP70 response to exercise in a similar manner to estrogen.

The expression of HSP70 in skeletal muscle, both under basal conditions and in response to exercise, is muscle fiber type specific. For example, basal expression of HSP70 is largely restricted to type I and type IIA muscle fibers in both rabbit tibialis anterior (27) and rat plantaris (30), with the highest levels actually found in type IIA fibers. Recently, we have shown that HSP70 protein expression in vastus lateralis of healthy men increases more rapidly and relatively more in type I fibers than type II fibers in response to fatiguing isometric exercise (46). Whether or not the potential effects of the ovarian sex hormones, estrogen and progesterone, on both basal HSP70 expression and the response to exercise in skeletal muscle are dependent on muscle fiber type have also not been investigated.

Hence the purpose of this study was to determine whether muscle fiber-type differences in basal HSP70 expression and
the HSP70 response to exercise are modulated by the ovarian sex hormones, estrogen and progesterone. We utilized an ovarioctomized rat model to manipulate estrogen and progesterone levels independently to test the hypotheses that both estrogen and progesterone would induce basal HSP70 expression and would attenuate the HSP70 response to damaging exercise in skeletal muscle. It was also hypothesized that the effects of ovarian sex hormones and exercise on HSP70 expression in muscle would be greater in type I muscle fibers than type II muscle fibers.

MATERIALS AND METHODS

Animals. This study was approved by the Animal Care Committee at Wilfrid Laurier University, and all procedures were performed in accordance with the Canada Council on Animal Care. A total of 48 ovarioctomized female Sprague-Dawley rats (Charles River Laboratories, LaSalle, Quebec) were utilized for this study. The ovaries were surgically removed at 9 wk of age, 1 wk before their arrival at the laboratory. Upon arrival, animals were housed two per cage in a controlled environment with constant temperature and humidity, with a standard 12:12-h light-dark cycle. All rats were fed Tekland 2215 Rodent Diet (Harland-Tekland, Madison, WI) and water ad libitum.

Experimental protocol. Animals were acclimatized for a period of 1 wk in the laboratory before being randomly assigned to one of three treatment groups (n = 16 per group): sham (S), progesterone (P), and estrogen (E). Estrogen (0.25 mg 17β-estradiol, 21-day time release pellet; Innovative Research of America, Sarasota, FL) and progesterone (25 mg progesterone, 21-day time release pellet, Innovative Research of America) supplementation were administered via subcutaneous pellet implantation (6, 43). Briefly, animals were anesthetized via inhaled isoflurane, and a small incision was made in the skinfolds of the neck. Blunt dissection was used to separate the skin from the underlying fascia. For estrogen- and progesterone-supplemented animals, the time-release pellet was inserted under the skin ~1 cm from the site of incision using forceps. For the sham procedure, blunt dissection and insertion of the forceps were performed without pellet insertion. Following each procedure, the incision was sealed using one to two drops of Vetbond (3M, St. Paul, MN). Animals were returned to their cages and allowed to recover for 8 days before beginning the exercise protocol. We have demonstrated previously that 1 wk of estrogen exposure in this model is sufficient to allow estrogen effects (16).

Each treatment group was then further subdivided into control (CTL) and exercised (EX) groups (n = 8). The exercise protocol consisted of eighteen 5-min running bouts, separated by 2-min rest periods. Animals ran downhill (~13.5°) at 17 m/min for a total time of 90 min, as previously detailed in Komulainen et al. (12). This is a nonfatiguing protocol, which has been shown to illicit significant damage and neutrophil infiltration in the soleus muscle (16, 45). Animals were killed 24 h following the exercise protocol using a lethal injection of pentobarbital sodium (55 mg/kg). Nonexercised (CTL) animals were euthanized at the same time as the EX animals. Animals were of the age of young adults (12 wk) at the time of death. Blood was extracted from the descending aorta and stored at ~80°C as plasma. The soleus from each leg was removed, frozen immediately in liquid nitrogen, and stored at ~80°C to be used for Western blot analysis. The other soleus was coated with optimal cutting temperature medium (Tissue-Tek, Torrance, CA) and quickly frozen in isopentane precooled in liquid nitrogen and stored at ~80°C until immunohistochemical analysis.

Serum analysis. Serum estrogen and progesterone levels were determined using commercially available radioimmunoassay kits (Coat-a-Count, Inter Medico, Markham, ON).

Western blot analysis. Western blotting was performed to determine the relative expression levels of HSP70 in soleus from S-, P-, and E-treated rats from both CTL and EX groups. After ensuring linearity of band density, samples (5 μg total protein) were applied to 10% polyacrylamide gels, and proteins were separated using standard SDS-PAGE protocols (14) and then transferred to polyvinylidene difluoride membranes (BioRad, Hercules, CA). A biotinylated ladder was used as a molecular mass standard (Cell Signaling Technology, Beverly, MA). Before applying the primary antibodies, the membranes were cut horizontally to separate proteins of molecular mass above and below ~60 kDa to separate the HSP70 (~70 kDa) protein band from the α-actin protein band (~42 kDa), which was used to normalize the HSP70 signal to control for protein loading. After blocking with a 10% skim milk suspension, the membranes containing the higher molecular mass proteins (i.e., above ~60 kDa) were incubated overnight at 4°C with anti-HSP70 monoclonal antibody (SPA-810, Assay Designs, Ann Arbor, MI), and the membranes containing the lower molecular mass proteins (i.e., below ~60 kDa) were incubated for 1 h with anti-α-sarcomeric actin antibody 5C5 (Sigma). Membranes were washed in Tris-buffered saline containing 0.1% Tween 20 and treated with horseradish peroxidase-conjugated anti-mouse secondary antibody (Santa Cruz Biotechnology). The membranes were washed once more, and the signals were detected with an enhanced chemiluminescence kit (GE healthcare) using a bioimaging system. The densitometric analysis of the HSP70 protein band was performed using GeneSnap software (Syngene). All samples were run in duplicate on separate gels, and HSP70 content was expressed relative to α-actin in arbitrary units.

Immunohistochemistry. Serial cross sections of the mounted soleus muscles (10 μm) were cut in a cryostat maintained at ~20°C. HSP70 immunohistochemistry was carried out according to the procedures described by Neufer et al. (27) with slight modifications, as described previously by our laboratory (46). Briefly, frozen muscle sections were air dried and then fixed in a 100% cold acetone solution for 10 min, washed in PBS (10 mM, pH 7.2), and permeabilized using 0.5% Triton X-100. After another wash in PBS, endogenous peroxidase was quenched by 0.6% H2O2. Following another wash, sections were blocked for 30 min in 5% horse serum at room temperature. The HSP70 primary monoclonal antibody (SPA-810, 1:200 dilution) was applied to the sections for 1 h at room temperature. After the sections were washed in PBS, biotinylated horse anti-mouse immunoglobulin G (Vector Laboratories) was applied for 30 min at room temperature. Following another rinse in PBS, the sections were incubated for 30 min with a 1:500 dilution of an alkaline phosphatase-streptavidin conjugate (Vector Laboratories). HSP70 antibody binding was visualized using an alkaline phosphatase secondary detection system (NovaRED substrate kit, Vector Laboratories), which produces a brown-red precipitate. To determine fiber-type-specific HSP70 expression, serial cross sections were stained for myosin ATPase activity using preincubation pH values of 4.55 and 10.3 (2). Randomly chosen fibers selected from the myosin ATPase stains (n = 10 for fiber types I and II) were matched with corresponding serial sections stained for HSP70 via a microscope (Nikon) linked to accompanying computer-based imaging analysis software (Image-Pro PLUS). Optical densities of the HSP70 stain was determined by subtracting the negative background (no primary antibody) and the background of the slide that was incubated with the primary antibody from the corresponding HSP70-positive serial section and expressed in arbitrary (blue-scale) units.

Statistical analyses. A two-way ANOVA was employed to analyze the treatment-specific (S vs. P vs. E) changes in HSP70 in response to exercise (CTL vs. EX) assessed in whole homogenates by Western blot analysis. A three-way ANOVA was employed to determine specific fiber-type differences (type I vs. type II) in HSP70 expression...
in response to ovarian sex hormone treatment (S vs. P vs. E) and in response to exercise (CTL vs. EX). Planned comparisons were also utilized to examine the independent effects of ovarian sex hormones on basal HSP70 expression (one-way ANOVA) and HSP70 response to exercise (paired \( t \)-test) in type I and type II fibers. The significance level was set at 0.05, and, when appropriate, a Newman-Keuls post hoc test was used to compare specific means (Statistica 5.0). Values are means (SD).

**RESULTS**

**Serum sex hormone levels and animal weights.** The body weights of all treatment groups before receiving hormone or sham treatment (Pre) and the final weights at time of death (Post) are shown in Table 1. There were no differences (\( P > 0.05 \)) in body weight between any of the groups at Pre, whereas, at Post, body weight was lower (\( P < 0.05 \)) in E compared with both S and P. There were no differences (\( P > 0.05 \)) in body weight between S and P at Post. The lower body weights observed for E relative to S are in accordance with previous studies using ovariectomized rats (6, 26, 42). The plasma concentrations of estradiol and progesterone of all treatment groups are also shown in Table 1. The data from CTL and EX animals were pooled, since no differences in serum estradiol or progesterone were evident between control and exercised conditions. As expected, plasma estradiol levels (pg/ml) were higher (\( P < 0.05 \)) in E, while progesterone levels (ng/ml) were higher (\( P < 0.05 \)) in P compared with the other groups.

**Western blotting analysis.** Western blotting was performed to determine the relative expression levels of HSP70 in soleus from S-, P-, and E-treated rats from both CTL and EX groups (Fig. 1). The HSP70 content in soleus from CTL animals was higher (\( P < 0.05 \)) in E compared with both S and P. Compared with CTL, HSP70 content in soleus was increased (\( P < 0.05 \)) at 24 h postexercise in both S and P, but not (\( P > 0.05 \)) in E. Following exercise, HSP70 content in soleus was not different (\( P > 0.05 \)) between any of the hormone treatment groups.

**Immunohistochemical analysis.** Muscle fiber typing revealed no differences in the percentage of type I (\( \sim 81\% \)) and type II (\( \sim 19\% \)) fibers in soleus muscle between any of the groups (data not shown) (Fig. 2). We did not try to distinguish between pure type IIA fibers [i.e., only expressing myosin heavy chain (MHC) IIA] and hybrid type IIC fibers expressing both MHC IIA and MHC I, which normally each account for \( \sim 9–10\% \) of the total fiber population of rat soleus (37). Our analyses of HSP70 content in soleus using a three-way ANOVA revealed a significant interaction between hormone treatment group and fiber type that was independent of exercise, and, therefore, the data from the CTL and EX animals were pooled (Fig. 3). Estrogen supplementation increased (\( P < 0.05 \)) HSP70 content in type I soleus fibers by \( \sim 63\% \), compared with S. HSP70 content in type II soleus fibers was also \( \sim 25\% \) higher in E compared with S, but this was not significant (\( P > 0.05 \)). There were no differences (\( P > 0.05 \)) in HSP70 content in either type II fiber between S and P. We also found a fiber-type-specific HSP70 response to exercise that was independent of hormone treatment, and, therefore, the data from hormone treatment groups were pooled (Fig. 4). Overall, HSP70 content was \( \sim 58\% \) higher (\( P < 0.05 \)) in type I soleus fibers and tended to be higher (\( P = 0.08 \)) in type II soleus fibers (\( \sim 31\% \)) following exercise compared with CTL. HSP70 content was more than twofold higher (\( P < 0.05 \)) in type I fibers than type II fibers in all groups (Figs. 3 and 4).

In this study, we wanted to examine the effects of ovarian sex hormones on fiber-type-specific basal HSP70 expression and HSP70 response to exercise in rat soleus. Table 2 displays the mean optical density values of the HSP70 stain for all individual groups and the results of our planned comparisons between CTL groups and within hormone treatment groups for both type I and type II fibers. The planned comparisons between CTL groups showed that estrogen supplementation increased (\( P < 0.05 \)) basal HSP70 content in both type I (\( \sim 2.3\text{-fold} \)) and type II fibers (\( \sim 1.6\text{-fold} \)), but the effect was more pronounced in type I fibers. As a result, the fiber-type differences in basal HSP70 content were also more pronounced in E compared with S with \( \sim 2.7\text{-} \) and 1.9-fold higher (\( P < 0.05 \)) levels in type I vs. type II fibers, respectively. Progesterone supplementation had no effect (\( P > 0.05 \)) on basal HSP70 content in either type I or type II fibers compared with

![Fig. 1. Effects of ovarian sex hormones and downhill running on 70-kDa heat shock protein (HSP70) expression in rat soleus determined by Western blotting.](image)

![Fig. 2. Representative Western blot of control (CTL) and exercised (EX) animal from each hormone treatment group [sham (S), progesterone (P), and estrogen (E)]. Staining with antibody 5C5 against α-actin was used as a control for protein loading (not shown).](image)

Table 1. Animal weight before and after hormone or sham treatment and plasma hormone (estradiol and progesterone) levels

<table>
<thead>
<tr>
<th></th>
<th>Pre, g</th>
<th>Post, g</th>
<th>Estradiol, pg/ml</th>
<th>Progesterone, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>273 (24)</td>
<td>290 (19)</td>
<td>18.9 (9)</td>
<td>1.07 (0.78)</td>
</tr>
<tr>
<td>Progesterone</td>
<td>275 (21)</td>
<td>293 (20)</td>
<td>16.4 (6)</td>
<td>3.50 (3.01)*</td>
</tr>
<tr>
<td>Estrogen</td>
<td>273 (17)</td>
<td>262 (16)*</td>
<td>153.0 (135)*</td>
<td>1.41 (1.31)</td>
</tr>
</tbody>
</table>

Values are means (SD); \( n = 16 \) rats/group. Pre, before treatment; Post, after treatment. *Values are significantly different from other treatment groups (\( P < 0.05 \)).
Planned comparisons within hormone treatment groups showed that exercise resulted in ~2.1-fold and ~1.6-fold increases ($P < 0.05$) in HSP70 content in type I and type II fibers, respectively, in both S and P. However, HSP70 content was not significantly increased ($P > 0.05$) by exercise, in either type I or type II fibers, in E. As a result, HSP70 content in both type I and type II fibers in EX animals was similar in all hormone treatment groups.

**DISCUSSION**

In this study, an ovariectomized rat model was employed to examine the effects of the ovarian sex hormones, estrogen and progesterone, on HSP70 expression in skeletal muscle under basal conditions and in response to damaging exercise. We used immunohistochemistry to determine whether the effects of ovarian sex hormones and exercise on HSP70 expression in muscle are fiber type dependent. As reported previously for cardiac muscle (8, 11, 47), here we show that estrogen increases basal HSP70 expression in rat soleus. Exercise-induced
HSP70 expression in rat soleus was diminished in estrogen-supplemented animals, confirming findings from previous studies that examined the HSP70 response to exercise in rat vastus (31), gastrocnemius (33), and heart (32). In contrast, we found that progesterone had no effects on basal HSP70 expression or the HSP70 response to exercise in rat soleus. Immunohistochemistry showed that the relative increase in basal HSP70 expression seen with estrogen and the relative increase in HSP70 expression seen with exercise in the absence of estrogen was 30–40% higher in type I fibers than type II fibers.

In accordance with our laboratory’s previous studies (39, 43), serum estrogen concentration in the E animals was approximately eight- to ninefold higher compared with both S and P animals and was at the high end of normal for rodents (23, 39, 52). The E animals were significantly lighter than both S and P animals, which is believed to be due to differences in insulin-like growth factor-1 and growth hormone release, which are downregulated by estrogen (7, 39). Serum progesterone levels in the P animals were approximately two- to threefold higher compared with S and E animals and were on the lower end of the physiological range (i.e., 2–46 ng/ml) in rats (3). Knowlton and Sun (11) demonstrated that progesterone induces HSP70 expression in rat cardiac myocytes in a concentration-dependent manner, with the greatest expression seen at higher concentrations of progesterone. However, it is possible that higher progesterone levels than were seen in the P animals are required to elicit changes in HSP70 in skeletal muscle.

This is the first study to show that estrogen increases basal HSP70 expression in skeletal muscle. Moreover, we established that both type I and type II fibers respond to estrogen with increased basal HSP70 expression, but the response in type I fibers is more pronounced. These results suggest that estrogen contributes to endogenous fiber-type differences in basal HSP70 expression, at least in the soleus muscle of female rats, which we found to be highest in type I fibers under all conditions. As noted in the Introduction, an earlier study reported that constitutive HSP70 levels in rat plantaris were highest in type IIA fibers, but in that study male rats were employed (30). Overall, our results would seem to contradict previous literature on sex differences, which reported either no difference in HSP70 levels in fast-twitch muscle between male and female rats (28), or HSP70 levels in rat soleus that were even higher in males compared with females (47). Importantly, we used an ovariectomized rat model, which allows for assessment of the independent effects of estrogen on HSP70 expression, whereas sex differences in basal HSP70 expression in skeletal muscle could be due to other factors known to influence HSP70 expression, such as testosterone (25). In the only other study to examine the effects of estrogen on skeletal muscle HSP70 expression in ovariectomized rats, basal HSP70 expression was not reported (33). A limitation of the present study is that an intact female group was not included as a control. Although ovary removal reduces cardiac HSP70 expression and estrogen replacement therapy maintains cardiac HSP70 at the level of intact females (47), it is unknown if the same would be true in skeletal muscle.

The effects of estrogen on gene expression are mediated by intracellular estrogen receptors (ERs), which are ligand-activated transcription factors with the ability to activate transcription from estrogen response elements (13). However, at least in cardiomyocytes, regulation of HSP70 expression by estrogen appears to be mediated by a nongenomic pathway, involving activation of nuclear factor-κB and HSF-1 (8, 38). There are two known ERs, ER-α and ER-β, that are both expressed in skeletal muscle (1, 49). The content and distribution of ER isoforms among skeletal muscle fiber types have not been quantified specifically; however, results from two independent studies, one in rats (15) and one in humans (49), suggest that ERs may be expressed more highly in type I fibers than type II fibers. Lemoine et al. (15) showed that ER-α mRNA was significantly higher in soleus compared with gastrocnemius and extensor digitorum longus in female rats, and Wiik et al. (49) demonstrated a significant positive correlation between ER-β mRNA levels and type I fiber percentage in humans. Thus it stands to reason that the fiber-type differences in basal HSP70 we observed with respect to estrogen supplementation could be accounted for by differences in the concentration of ERs between type I and type II fibers.

A major objective of this study was to examine the interactive effects of ovarian sex hormones and exercise on fiber-type-specific HSP70 expression in rat soleus. To date, sex differences and the effects of ovarian sex hormones on exercise-induced HSP70 expression in skeletal muscle have only been assessed at the whole muscle level using Western blotting (31, 33). In each of these studies, estrogen was shown to inhibit the ability of acute exercise to increase the muscle HSP70 content above basal levels. In agreement with these studies, we found that HSP70 content in soleus was increased above control in response to downhill running in both S and P animals, but not in E animals. The fact that the HSP70 response to exercise was almost identical in S and P animals provides further support that progesterone alone does not regulate HSP70 expression in skeletal muscle or that progesterone

### Table 2. Planned comparisons between hormone treatment groups and between control and exercise groups showing differences in fiber-type-specific HSP70 content in soleus

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Exercise</th>
<th>Control</th>
<th>Exercise</th>
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<tbody>
<tr>
<td>sham</td>
<td>558.2 (132.6)</td>
<td>1,186.9 (440.1)†</td>
<td>294.6 (59.2)</td>
<td>519.0 (190.6)†</td>
</tr>
<tr>
<td>progesterone</td>
<td>601.4 (226.3)</td>
<td>1,281.9 (447.2)†</td>
<td>322.6 (50.4)</td>
<td>481.9 (155.5)†</td>
</tr>
<tr>
<td>estrogen</td>
<td>1,287 (523.3)†</td>
<td>1,568.2 (348.6)</td>
<td>469.0 (190.6)†</td>
<td>569.6 (230.1)</td>
</tr>
</tbody>
</table>

Values are means (SD) in arbitrary units; *Values are significantly different from other hormone treatment groups (P < 0.05). †Values are significantly different from control (P < 0.05).
supplementation in this study was less than needed to elicit a response. Our fiber-type analyses showed that estrogen attenuated the HSP70 response to exercise equally in type I and type II fibers, whereas, in both S and P animals, the exercise-induced increase in HSP70 was more marked in type I fibers. This latter finding is in accordance with our laboratory’s previous work (46), where we found that the HSP70 response to acute isometric exercise in human vastus lateralis was more rapid and more robust in type I fibers than type II fibers.

The downhill running protocol employed in this study has been shown to induce structural protein damage (17), oxidative damage as indicated by protein carbonyl formation in soleus muscle (51), disruption of the membrane systems involved in excitation-contraction coupling (39), neutrophil and macrophage infiltration (5, 45), and muscle satellite cell proliferation (6). Comparisons between fiber types within the same muscle indicate that type II fibers sustain greater eccentric damage than type I fibers (40). Therefore, if eccentric muscle damage was the primary stimulus for HSP70 induction in response to our downhill running protocol, we would have expected larger exercise-induced increases in HSP70 content in type II compared with type I fibers. Alternatively, it is likely that type I fibers were recruited more than type II fibers in rat soleus during downhill running, which may provide increased stimulus for HSP70 production in type I fibers. Differences in motor unit recruitment patterns within the type I fiber pool both at rest and during exercise might also explain why some type I fibers stain darker (i.e., express more HSP70) than others.

Estrogen has been shown to reduce postexercise damage and inflammation in rat soleus (5, 6) by a non-ER-mediated process, probably owing to its antioxidant and membrane stabilizing actions (44). Thus estrogen may reduce the intracellular signals associated with exercise that are involved in activating the HSP70 response. On the other hand, the blunted HSP70 response to exercise observed in the estrogen-supplemented animals may be related to the high initial (basal) levels of HSP70 already present in the muscle before exercise, which should reduce exercise-induced damage (22) and minimize the need for further HSP70 production. If this is true, then estrogen should be able to protect against exercise-induced muscle damage more in type I fibers than type II fibers. It is unknown if the ability of estrogen to prevent exercise-induced muscle damage is fiber-type specific; however, estrogen does attenuate postexercise leukocyte infiltration into predominantly red (soleus) and white (white vastus) muscle to the same degree (5, 9). Finally, given the differences in body weight between the treatment groups, we cannot rule out the possibility that the physiological strain imposed by exercise was lower in E animals compared with both S and P animals, possibly resulting in lower core and muscle temperatures and/or metabolic stresses, which could explain the attenuated exercise-induced HSP70 response in E.

The ability of downhill running (i.e., eccentric exercise) to increase HSP70 content in rat soleus in S and P animals is consistent with human studies that showed HSP70 mRNA and protein were upregulated in skeletal muscle following exercise involving damaging lengthening contractions (20, 41). It is important to note that, in the study by Thompson et al., the participants included four men and four women, and it was reported that HSP70 content in biceps brachii, a mixed muscle containing ~50% type I and 50% type II fibers (24), was increased at 48 h following the exercise protocol in every participant. Unfortunately, specific sex comparisons were not reported in that study, so it is unknown if there were differences between men and women in the magnitude of the HSP70 response. Furthermore, it is unknown if these authors controlled for the estrus cycle, which may influence the HSP70 response to exercise (29). Also, our study only examined the independent effects of both estrogen and progesterone on skeletal muscle HSP70 expression, and the combined effects of these ovarian sex hormones at physiological concentrations remain unknown. Future studies should investigate the combined effects of estrogen and progesterone on regulation of HSP70 expression in skeletal muscle and whether sex influences HSP70 expression in human skeletal muscle under basal conditions and in response to exercise.

In summary, we have found that HSP70 expression in soleus from ovarioctomized rats increases in response to downhill running, an effect that is more pronounced in type I fibers compared with type II fibers. Supplementation with estrogen increases basal HSP70 expression and prevents further increases in HSP70 in response to exercise in rat soleus. The effects of estrogen on basal HSP70 expression in rat soleus were more pronounced in type I fibers compared with type II fibers, whereas the effects of estrogen on the HSP70 response to exercise were independent of muscle fiber type. Neither basal HSP70 expression nor the HSP70 response to exercise was altered by progesterone supplementation. This study contributes to a growing body of work supporting the view that estrogen regulates HSP70 expression in muscle.

GRANTS

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