Sexual dimorphism of the intracellular heat shock protein 72 response

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Nickerson, M., S. L. Kennedy, J. D. Johnson, and M. Fleshner. Sexual dimorphism of the intracellular heat shock protein 72 response. J Appl Physiol 101: 566–575, 2006. First published May 11, 2006; doi:10.1152/japplphysiol.00259.2006.—The majority of previous work examining stress responses has been done in males. Recently, it has become clear that the impact of stressor exposure is modulated by sex. One stress response that may be affected by sex is the induction of intracellular heat shock protein (HSP) 72, which is a stress-responsive molecular chaperone that refolds denatured proteins and promotes cellular survival. The following study compared HSP72 in males and females and also examined whether the estrous cycle altered HSP72 induction in females. We hypothesized that females compared with males would have a constrained HSP72 response after an acute stressor and that the stress-induced HSP72 response in females would fluctuate with the estrous cycle. Male and female F344 rats were either left in their home cage or exposed to acute tail-shock stress (8–10/group). Immediately following stressor, trunk blood was collected and tissues were flash frozen. Vaginal smear and estrogen enzyme immunoassay were used to categorize the phase of estrous. Results show that female rats had a greater corticosterone response than males, that both males and females exhibit a stress-induced release of progesterone, and that males and females had equal levels of stress-induced circulating norepinephrine. Sexual dimorphism of the HSP72 (ELISA) response existed in pituitary gland, mesenteric lymph nodes, and liver such that female rats had an attenuated HSP72 response compared with males after stress. The adrenal glands, spleen, lymph nodes, and liver did not exhibit sexual dimorphism of the HSP72 response. The estrous cycle did not have a significant effect on basal or stress-induced HSP72 in females.

HEAT SHOCK PROTEIN (HSP) 72 is a 72-kDa member of the HSP family that is upregulated intracellularly in response to various stressors (8, 18, 29, 36, 50, 58). The induction of HSP72 enhances cellular survival following stress (9, 32, 112). Our laboratory previously reported that male Fisher 344 rats exhibit a robust induction of HSP72 in many stress-responsive tissues, including the pituitary gland, adrenal glands, mesenteric lymph nodes, spleen, and liver (11, 66). Although there is some evidence for a unique HSP72 response in female rats compared with male rats (72, 73, 107), the female HSP72 response has not been broadly characterized. There are several potential induction signals for HSP72, such as the oxidative stress cascade and the release of stress hormones (i.e., corticosterone and norepinephrine). It is possible that estrogen alters the impact or the downstream interactions of potential induction signals. Therefore, this study has two purposes: 1) to determine whether there is a sexual dimorphism in the HSP72 response in various stress-responsive tissues, and 2) to determine whether the estrous cycle affects the HSP72 response in females.

There are two possible ways that estrogen might be involved in the HSP72 response. The first is by directly protecting cells from damage, thus reducing the need for HSP72. The second is by estrogen interacting with the transcription of HSP72 by altering cellular processes. Estrogen has protective effects independently of HSP72 that might decrease the damage accrued by a cell during cellular stress (30). If cellular damage does ensue, estrogen may then be able to facilitate the induction of HSP72. Traditionally, the main sites recognized as estrogen targets are bone and reproductive organs, and the effect of estrogen on these tissues is for reproductive or developmental purposes. Studies have demonstrated that estrogen can affect several additional tissues, and a role for estrogen has been postulated in the pituitary gland (48, 53), adrenal glands (53, 110), mesenteric lymph nodes (20, 85), spleen (87, 90, 91), liver (25, 54), heart (71, 79), and muscle (4, 28). Although estrogen is largely recognized as a reproductive hormone, it can also protect various cell types during acute stress. For example, in vitro pretreatment with estrogen protects endothelial progenitor cells (41), neurons (2, 113), articular chondrocytes (19), and human lens epithelial cells (109) from acute oxidative stress. In addition, estrogen can be a key protective feature of the in vivo female stress response and may buffer tissues from the impact of stressors, thus preventing damage during exposure to an acute stressor (44, 67, 82, 95, 113). Females with either intact ovaries or estrogen replacement fare better than their ovariectomized counterparts when exposed to a variety of stressors, including exercise-heat stress (65), oxidative stressors (95), cerebral ischemia (70, 88), neuropathic pain (100), and cardiac stressors (22, 59). Interestingly, estrogen protects cells against similar stressors from which HSP72 protects cells (8, 17, 29, 51, 58).

Both in vitro and in vivo evidence support a link between estrogen and HSP72. For example, in vitro studies using cardiac myocytes have shown that estrogen can induce the HSP72 transcription factor, heat shock factor 1 (49). In addition, estrogen pretreatment of cardiac myogenic cells results in upregulation of both estrogen receptor and HSP72, leading to elevated protection from lethal heat stress (1). Hamilton et al. further demonstrated that estrogen added to culture of human foreskin fibroblasts interacts with and activates heat shock factor, leading to upregulation of HSP72 and enhanced cell survival (36). There has been little in vivo work done to determine whether males and females have differential expression of HSP72 in peripheral stress-involved organs, including the pituitary gland, adrenal glands, mesenteric lymph nodes, spleen, liver, left ventricle of the heart, and skeletal muscle. A few studies, however, suggest that there is a sexual dimorphism of the HSP72 response in some tissues. Papadimitriou et al.
(73) demonstrated that activated estrogen receptors enhance uterine HSP72, and Paroo et al. (75) reported that females have lower cardiac HSP72 levels compared with males following a single bout of forced exercise and that this effect is likely mediated by estrogen. Also, during the aging process, postmenopausal females exhibit a decrease in estrogen production and are more susceptible to the negative aspects of exposure to acute stressors (45, 60, 86), which may be related to an impairment in the ability of aged organisms to express intracellular HSP72 (93, 106). It is possible that it is the lack of estrogen in aged animals that leads to an impaired intracellular HSP72 response. All of this evidence has led us to investigate how the female hormone profile might affect the induction of HSP72. Thus the purpose of the first part of our two-part study was to compare the intracellular HSP72 response of males and females in various stress-responsive tissues following an acute stressor.

The second part of our study involved only female rats and was designed to understand how different stages of the estrous cycle might affect the expression of intracellular HSP72 in response to an acute stressor. During proestrus, estrogen levels are the highest, whereas during estrus, estrogen levels are the lowest. During diestrus, estrogen levels slowly rise as the cycle reenters proestrus. It has been shown that hormonal variations across the estrous cycle can affect behavioral manifestations of learned helplessness (42), psychosocial stress responses (45), and cellular processes in tissues throughout the body (3, 37, 78, 80, 102). We tested, therefore, whether the minor fluctuations of estrogen across the estrous cycle are sufficient to affect the intracellular protein HSP72 in various stress-responsive tissues.

The current experiment is important because such work will allow for a more global understanding of the induction of HSP72 in males and females, as well as a characterization of the female HSP72 response across the estrous cycle. We hypothesized that basal levels of HSP72 would not differ between males and females and that both males and females would experience a robust induction of HSP72 after an acute stressor. We further hypothesized that males would induce more HSP72 than females immediately following stressor exposure. The presence of estrogen in females may offer some initial protection for cells against stress and therefore result in an attenuated drive on the female HSP72 system. Males and females may have distinct mechanisms for altering HSP72 induction, whereas females at all stages of estrus should have the same mechanism for modulating HSP72.

All cycling females have significant levels of estrogen compared with males, but levels of estrogen fluctuate during the estrous cycle. Thus we hypothesized that the natural fluctuations of estrogen throughout the estrous cycle might be sufficient to modulate the intracellular HSP72 response to an acute stressor. Specifically, we predicted that during proestrus, when estrogen levels are the highest, stress-induced HSP72 would be at its lowest, and that during estrus, when estrogen levels are lowest, stress-induced HSP72 would be at its highest. Circulating hormones, including estrogen, progesterone, corticosterone, and norepinephrine, were measured to verify accuracy of vaginal smears and to measure activation of the systemic stress response. Intracellular HSP72 content was measured across a range of tissues to determine any potential tissue-specific sexual dimorphisms or changes across the estrous cycle in the cellular stress response.

**METHODS**

**Housing**

Male (n = 43; 215–250 g) and female (n = 78; 145–175 g) rats were individually housed in Plexiglas cages (60 × 30 × 24 cm) with food and water available ad libitum. Colonies were maintained in a pathogen-free barrier facility with a 12:12-h light-dark cycle. Animals were allowed to acclimate for 2 wk before experimentation. The University of Colorado Institutional Animal Care and Use Committee approved all animal procedures.

**Tail-Shock Stress Protocol**

Animals either remained undisturbed in their home cage (HCC) or were exposed to inescapable tail-shock stress (IS). IS animals were put into Plexiglas restraining tubes with their tails protruding. Contact beams were then placed across their tails, ~2 in. apart, and the rats were then exposed to 100 1.6-mA tail shocks (5-s duration, variable intertrial interval averaging 60 s). The stressor, which has both psychological and physical components, lasts 100 min and has been previously documented to robustly induce HSP72 (11).

**Decapitation and Dissection**

Immediately following stressor termination, rats were rapidly decapitated, and vaginal smears were performed; a vaginal lavage (~200 μl of saline) was used to collect cells. The sample of cells was then put onto a glass slide and viewed under a microscope at a magnification of ×100. The phases of the 4-day rat estrous cycle can be determined using a vaginal smear, and in the present study we delineated between proestrus, estrus, and diestrus. Phase of estrus was determined by cell type, and animals were categorized into one of three groups: 1) proestrus was defined by a predominance of round, nucleated epithelial cells; 2) estrus was defined by the presence of cornified squamous epithelial cells that were often clustered; 3) diestrus was defined by a prevalence of small leukocytes and few epithelial cells. Pituitary, adrenal, mesenteric lymph nodes, spleen, liver, left ventricle of the heart, and triceps muscle were quickly dissected, put into 1.5-ml Eppendorf snap cap tubes, and flash frozen in liquid nitrogen. The tissue samples were stored at −80°C until sonication.

**Treatment of Blood**

Immediately after stressor termination and rapid decapitation, two aliquots of trunk blood were collected: one tube contained glutathione to prevent catecholamine oxidation, and the second tube contained EDTA to prevent clotting. The serum with glutathione was used for catecholamine assessment, and the plasma collected on EDTA was used for corticosterone, 17β-estradiol, and progesterone measurements. Blood was spun at 2,000 rpm in a refrigerated centrifuge for 15 min. After centrifugation, plasma and serum samples were aliquoted and stored at −80°C until time of assay. Serum samples from females were measured for 17β-estradiol using an EIA (Cayman Chemicals, Ann Arbor, MI), and progesterone was measured in both males and females using ELISA (R&D Systems, Minneapolis, MN). Corticosterone was measured using RIA (IMP Biomedicals, Orangeburg, NY). All assays were performed per manufacturers’ instructions.

**Catecholamine content.** Catecholamine content was determined using HPLC (ESA 501 Coulouche II with 582 solvent delivery unit and 542 autosampler) as previously described (62). Catecholamines were driven onto alumina with 1.5 M Tris buffer, pH 8.6, in 2% EDTA, and vortexed for 10 min. The alumina was washed with 3 ml of distilled water and vortexed for 5 min. Catecholamines were then extracted with 0.1 M HClO4 and vortexed a final time for 10 min. The
serum extract was then filtered through a 0.22-μm syringe filter (Osmonics, Minnetonka, MN), and 20 μl were injected in duplicate onto the HPLC column (ESA MD-150 for tissues; ESA HR-80 for plasma) carried by mobile phase consisting of acetonitrile, phosphate buffer, and an ion-pairing agent. Data were integrated using the ESA 501 chromatography system. Serum data are presented as picograms norepinephrine per milliliter.

**Tissue Processing and HSP72 Measurements**

Sonication buffer was added to each tissue sample. The sonication buffer was composed of extraction buffer, enzyme cocktail, and PMSF. The extraction buffer was Iscove’s medium (Gibco 12440-020; Coon Rapids, MN) with 5% fetal calf serum (Gibco 16000-044). The ×10 enzyme cocktail was 13.1% 1 M amino-N-caproic acid (Sigma-A-7824; St. Louis, MO), 3.722% 100 mM EDTA (Sigma, E-5134), 0.783% 50 mM benzamidine (Sigma B-6506), and 82.485% double-distilled water (ddH2O). A ration of 0.348% 20 mM PMSF (Sigma P-7626) was dissolved in isopropanol. Sonication volumes were as follows: adrenal glands = 500 μl, one-third of spleen = 1 ml, left ventricle of heart = 600 μl, mesenteric lymph nodes = 500 μl, pituitary gland = 250 μl, small piece of liver = 1 ml, belly of triceps muscle = 1 ml. Sonication was performed in the original storage tubes with a Fisher sonic dismembrator, model 100 (Fisher Scientific, Pittsburgh, PA). All samples were kept on ice before and after sonication. Immediately after sonication, samples were spun at 4°C for 10 min at 10,000 rpm. Supernatant was carefully pipetted into new 1.5-ml snap caps and stored at 4°C for <24 h until ELISA procedure. Homogenates were analyzed using HSP72 ELISA (Stressgen, Victoria, Canada), which were performed per manufacturer’s instructions, and some samples were diluted to obtain optimal concentrations for the ELISA. Adrenals were diluted 1:10, mesenteric lymph nodes were diluted 1:4, spleen was diluted 1:4, and liver was diluted 1:4; pituitary and left ventricle tissue sonicates were not diluted. Total protein was measured using a Bradford assay to correct for total tissue assayed. Bradford assay tissue dilutions were made with ddH2O: adrenal glands (1:10), spleen (1:50), left ventricle of heart (1:100), mesenteric lymph nodes (1:40), pituitary gland (1:20), liver (1:50), and belly of triceps muscle (1:60). Final concentrations of HSP72 are expressed as picograms per microgram of protein.

**Statistics**

The differences between males and females were determined using a 2 (HCC, IS) × 2 (male, female) ANOVA. The main effects of the estrous cycle were determined by a 2 (HCC, IS) × 3 (proestrus, estrus, diestrus) ANOVA. When appropriate, post hoc analyses were calculated using the protected least significant difference.

**RESULTS**

**Male vs. Female**

**Circulating estrogen.** There was a significant increase in circulating estrogen in females following stressor exposure \( F(1,70) = 6.108, P = 0.0217 \) as shown in Fig. 1A.

**Circulating progesterone.** As shown in Fig. 1B, males and females both exhibited stress-induced progesterone, and there was a main effect of stress \( F(1,115) = 265.173, P = 0.0001 \). Females had more progesterone than males at baseline and following stress, resulting in a significant main effect of sex \( F(1,115) = 140.179, P < 0.0001 \). The stress × sex interaction was not significant.

**Circulating corticosterone.** Figure 1C shows that males and females both significantly induced corticosterone following stressor exposure \( F(1,69) = 196.116, P = 0.0001 \). Females induced significantly more corticosterone than males, which resulted in a main effect of sex \( F(1,69) = 27.234, P < 0.0001 \) and a significant stress × sex interaction \( F(1,69) = 22.183, P = 0.0001 \), which was due to a greater corticosterone response to stressor exposure in females than in males.

**Circulating norepinephrine.** Both males and females had significantly elevated circulating norepinephrine following acute tail-shock stress. There was a main effect of stress.
[F(1,56) = 37.534, \( P = 0.0001 \)], but there was not an effect of stress or a stress × sex interaction. This relationship is depicted in Fig. 1D.

**Triceps muscle.** Neither males nor females exhibited a significant induction of HSP72 in the triceps muscle following stressor exposure, and there was no effect of sex on baseline or stress-induced levels of triceps HSP72. The results are therefore not shown.

**Pituitary gland.** Figure 2A shows that stressor exposure resulted in a significant increase of HSP72 in the pituitary gland \([F(1,117) = 145.657, \ P < 0.0001]\). There was also a significant effect of sex \([F(1,117) = 18.621, \ P < 0.0001]\) such that females expressed significantly less HSP72 following stressor exposure than did males, and this resulted in a stress × sex interaction \([F(1,117) = 16.683, \ P < 0.0001]\). Post hoc analyses revealed that stress-induced HSP72 in the pituitary of females is significantly lower than in males \([F(3,114) = 79.305, \ P < 0.0001]\).

**Adrenal glands.** In the adrenal glands, stressor exposure resulted in a significant increase in HSP72 \([F(1,116) = 71.268, \ P < 0.0001]\), which is depicted in Fig. 2B. There was, however, no effect of sex on the expression of HSP72.

**Mesenteric lymph nodes.** As represented in Fig. 2C, there was a significant main effect of stressor exposure in the mesenteric lymph nodes \([F(1,76) = 84.937, \ P < 0.0001]\), and there was also a main effect of sex \([F(1,76) = 8.411, \ P < 0.05]\) such that females expressed less HSP72 than males. There was also a stress × sex interaction \([F(1,76) = 5.445, \ P < 0.05]\) in which females expressed significantly less stress-induced HSP72 than males. Post hoc analyses revealed that stress-induced HSP72 in the mesenteric lymph nodes of females is significantly lower than in males \([F(3,76) = 30.025, \ P < 0.001]\).

**Spleen.** Stress resulted in a significant increase of HSP72 in the spleen \([F(1,72) = 301.729, \ P < 0.0001]\), which is shown in Fig. 2D. There was neither a main effect of sex nor a stress × sex interaction on the expression of splenic HSP72.

**Liver.** Figure 2E shows that there was a main effect of stress \([F(1,115) = 165.893, \ P < 0.0001]\), a main effect of sex \([F(1,115) = 53.984, \ P < 0.0001]\), and a stress × sex interaction \([F(1,115) = 78.264, \ P < 0.0001]\) in the liver. Both males and females exhibited a significant increase of liver HSP72 following stressor exposure, but females expressed significantly less HSP72 than males. Post hoc analyses revealed that
stress-induced HSP72 in the liver of females was significantly lower than in males \([F(3,115) = 79.442, P < 0.0001]\).

**Left ventricle of the heart.** HSP72 levels in the left ventricle of the heart are portrayed in Fig. 2F, and there was a main effect of stress \([F(1,116) = 16.566, P < 0.0001]\). The increase of HSP72 following stressor exposure was unaffected by sex, and there was not a stress × sex interaction.

**Estrous Cycle**

Circulating estrogen. As shown in Fig. 3A, the main effect of stress neared significance \([F(1,68) = 2.066, P = 0.0684]\), and there was a main effect of cycle \([F(2,68) = 2.472, P = 0.05]\) such that females in proestrus had significantly more estrogen than animals in estrus \((P = 0.0089)\) or diestrus \((P = 0.0438)\).

Circulating progesterone. There was a significant main effect of stress \([F(1,69) = 137.179, P = 0.0001]\) on circulating levels of progesterone. Estrous phase did not significantly alter circulating progesterone. This is demonstrated in Fig. 3B.

Circulating corticosterone. Females had significantly elevated circulating corticosterone following stressor exposure, and there was a main effect of stress \([F(1,39) = 168.994, P = 0.0001]\) on levels of circulating corticosterone, which is shown in Fig. 3C. There was not a main effect of cycle, and there was not a stress × cycle interaction.

Circulating norepinephrine. Stressor exposure resulted in significantly elevated concentrations of circulating norepinephrine. There was a main effect of stress \([F(1,72) = 15.634, P = 0.0002]\). There was not a significant main effect of cycle \([F(2,72) = 1.827, P = 0.0697]\), and there was not a stress × cycle interaction, which is shown in Fig. 3D.

**Tissues**. There was a significant induction of HSP72 in all tissues examined \((P < 0.01)\). Estrous cycle did not affect HSP72 induction. Post hoc analyses done on stress-induced levels of HSP72 during each phase of the estrous cycle revealed that in nearly every instance HSP72 was induced compared with nonstressed animals during the same phase of estrous. The levels of HSP72 across the estrous cycle are shown in Fig. 4.

**DISCUSSION**

In this study, we compared the induction of HSP72 immediately after an acute stressor in male and female Fisher 344 rats and also examined the degree to which HSP72 was induced in female rats at different stages of the estrous cycle. Although this study is descriptive in nature, it fills an important gap in the current HSP72 literature. Both males and females exhibited similar baseline levels of HSP72, and acute stressor exposure resulted in significant induction of HSP72 in both males and females in the pituitary gland, adrenals, mesenteric lymph nodes, spleen, liver, and left ventricle of the heart, but not in the triceps muscle. Interestingly, stress-induced levels of HSP72 were significantly less in the pituitary, mesenteric lymph nodes, and liver of females compared with males. Estrous cycle did not significantly impact the basal or stress-induced levels of HSP72 in female rats in any tissue examined. These data suggest that the presence of estrogen in females compared with males is sufficient to influence the induction of HSP72 but that the minor fluctuations of estrogen that occur in females during the estrous cycle are insufficient to alter the induction of HSP72 in some stress-responsive tissues.

Estrogen was measured in females to validate vaginal smears and to show the degree to which ovarian hormones are expressed after an acute stressor in females. Baseline levels of estrogen confirmed our vaginal smear classifications; animals that were categorized based on vaginal cell morphology as being in proestrus had the highest levels of circulating estrogen, whereas animals categorized as being in estrus had the lowest levels of circulating estrogen. Progesterone was significantly elevated following stressor exposure in both males and females. Increases in plasma progesterone and estrogen following tail-shock stress have been reported previously (92), and these increases are likely due to the drive on steroidogenesis for the creation of corticosterone. Progesterone is a pre-

![Fig. 3. Effect of estrous cycle on circulating hormones.](image-url)
cursor to corticosterone, and estrogen is a by-product of a parallel pathway that is driven by high levels of progesterone.

We also measured circulating levels of the stress-related hormones corticosterone and norepinephrine, which have been implicated in the induction of HSP72 (1, 10, 39, 103, 104). However, the patterns of corticosterone and norepinephrine induction in males and females do not globally match the induction of HSP72 in males and females, suggesting that the sexual dimorphisms of the HSP72 response are not solely dependent on either of these stress hormones. Corticosterone is a measure of hypothalamic-pituitary-adrenal (HPA) axis activation. Activation of the HPA axis results in release of corticosterone from the adrenal gland. Circulating corticosterone can then affect tissues expressing the glucocorticoid or mineralocorticoid receptor. Among the tissues examined in the current experiment, substantial levels of glucocorticoid receptors are located in the pituitary gland (63, 69), adrenal gland (5, 76), liver (34, 57), and skeletal muscle (61). It is possible that alterations in circulating corticosterone can affect intracellular HSP72 induction. Our results show that basal corticosterone levels are the same between male and female rats; however, females show a much greater corticosterone response than males immediately following an acute stressor, which is supported by previous literature (7, 14, 26, 52). Because sexual dimorphisms of the HSP72 response do not directly parallel the presence of glucocorticoid receptors, it is unlikely that corticosterone mediates the attenuated HSP72 response in females following stressor exposure.

Both males and females exhibit a significant increase in circulating norepinephrine, which is an indirect measure of sympathetic nervous system (SNS) activity. Evidence for sexual dimorphisms of SNS activity have been reported at the level of the superior cervical ganglion (6) and in SNS control over metabolism (21, 40) or hypertension (83, 96). Unlike the general response of the HPA axis, however, the SNS is topographically organized so that it differentially innervates several tissues. Because of this specific organization and because of the dynamic nature of the adrenergic receptors, it has been difficult for researchers to pinpoint sexual dimorphisms of the SNS. There is, nonetheless, growing evidence for an interaction between estrogen and the adrenergic receptors, especially the $\alpha_2$-adrenergic receptor (22, 24, 46, 77, 97), but a study comparing the relative SNS innervation of peripheral tissues between males and females has not yet been performed. In the current experiment, the tissues that are classically recognized as receiving direct SNS innervation are the adrenal gland, spleen, liver, and heart (12, 47).

Fig. 4. Effect of estrous cycle on the HSP72 response in females. A: at all stages of the estrous cycle, HSP72 is significantly induced in the pituitary gland. B: there is a significant induction of HSP72 in the adrenal gland at all stages of the estrous cycle. C: HSP72 is equally induced during all phases of the estrous cycle in the mesenteric lymph nodes. D: splenic HSP72 is elevated to the same degree during all stages of the estrous cycle. E: HSP72 in the liver is equally elevated across the estrous cycle. F: HSP72 is significantly elevated during proestrus and diestrus in the heart. Values are means ± SE. *Significant effect of inescapable tail shock stress during the same phase of the estrous cycle ($P < 0.05$).
The results from the first part of our study allow us to delineate between three distinct patterns of tissue intracellular HSP72 expression: 1) nonresponsive tissues; 2) equally responsive tissues; 3) sexually dimorphic tissues. The triceps muscle is a nonresponding tissue; neither males nor females exhibited an induction of HSP72 following stressor exposure. This muscle was chosen because it expresses both oxidative and nonoxidative components, making it representative of skeletal muscles in general (for review of muscle HSP72, see Refs. 55, 56). There are many studies in which stress-induced HSP72 was detected in muscle (15, 98) and several studies in which stress-induced HSP72 was not detected in muscle (68, 81, 105). There is, however, no evidence in the literature that the triceps muscle of the rat expresses HSP72 after tail-shock stress. Although it is possible that the variability presented in the triceps muscle of the present experiment prevents us from revealing a clear HSP72 response or that HSP72 is induced at a later time point in skeletal muscle or that the triceps is not used during tail-shock stress and therefore fails to produce HSP72, we observed a lack of HSP72 induction in the triceps muscle of male or female rats.

The tissues in which we see an equivalent expression of HSP72 between males and females are the adrenal glands, spleen, and heart. These tissues have direct innervation by the SNS, and it is possible that the stress signal received by the adrenal glands, spleen, and heart is primarily driven by adrenergic mechanisms. Adrenergic signaling can affect intracellular signaling cascades like NF-centage (27, 33, 38) or local levels of reactive oxygen species due to the metabolism of norepinephrine (30, 31, 99) and, therefore, the expression of HSP72 (35, 89). There is a great deal of evidence that adrenergic receptor activation can either enhance or directly upregulate intracellular HSP72 (16, 64, 104). Adrenergic signaling could be such a potent mediator of the HSP72 that the induction of HSP72 is unaffected by the presence of estrogen and/or estrogen receptors in tissues that are directly innervated by the SNS. Spillover circulating norepinephrine levels are the same in males and females, which supports a similar overall drive of the SNS in males and females. There are several studies in humans that report constrained SNS activity in females (21, 23, 40, 43). Our study, however, uses rats and is a different model of stress and therefore does not refute or substantiate those previous reports in humans. There is a paucity of rodent literature describing sexual dimorphisms of the SNS response, which suggests that more work is required before firm conclusions can be made. It is possible that some tissues, however, do present a sexual dimorphism and that the sexual dimorphism is due to estrogen rather than a direct consequence of HPA or SNS activity.

There is a sexual dimorphism of the HSP72 response to an acute tail-shock stressor in the pituitary, mesenteric lymph nodes, and liver. Of these tissues, the mesenteric lymph nodes and pituitary may receive less direct or less dense SNS innervation than other tissues examined in this experiment (13, 84, 94). The liver, however, has a unique characteristic in that it is the primary site of estrogen metabolism (101) and consequently has very high estrogen exposure. It is possible that the relative intensity of the estrogen and SNS stress signals affect the induction of HSP72. Therefore, the mesenteric lymph nodes, pituitary, and liver are tissues where there may be a high estrogen-to-stress signal ratio, and it is more likely that the estrogen will have an impact on the HSP72 response. The presence of estrogen may affect these tissues by buffering them from the stressor. By preventing stress-induced damage of the cell, estrogen can reduce the drive on the HSP72 system, and females may thus exhibit a reduced HSP72 response compared with males in tissues that have a higher estrogen-to-stress signal ratio. On the other hand, the adrenal glands, spleen, and heart may receive a more immediate, robust signal from the SNS and have a low estrogen-to-stress signal ratio, and the induction of HSP72 is therefore unaffected by the presence of estrogen.

Although the pituitary, mesenteric lymph nodes and liver exhibit a sexual dimorphism of the HSP72 response, the estrous cycle does not alter stress-induced HSP72 in any tissues of female rats. This study involved large groups, yet a significant effect of the estrous cycle on HSP72 induction following an acute stressor was not detected. It is important to recognize, however, that the estrous cycle is a natural, homeostatic event in females. It is plausible that the effects of an intense acute stressor are more robust than the effects of the natural estrous cycle on intracellular levels of HSP72. The comparison of females to other females across the estrous cycle is subtler than the comparison of males and females. The comparison between males and females tests how the presence of an estrogen system affects the induction of HSP72, whereas the comparison between females across the estrous cycle tests how minor fluctuations of estrogen might affect the expression of HSP72. The primary peripheral estrogen receptor, ERα, fluctuates during the estrous cycle to accommodate changing levels of estrogen (108), and excess circulating free estrogen exposure is further buffered by steroid binding protein in the blood. The mirroring patterns of ERα to circulating estrogen and fluctuations of steroid binding proteins, therefore, may prevent the tissues from being exposed to fluctuating levels of free estrogen during the estrous cycle. There is also evidence that ERβ may be an important factor in the female cardiac HSP72 response (114). Greater manipulations of estrogen and estrogen receptors are necessary to separate the distinct roles of estrogen and estrogen receptors on the expression of stress-induced intracellular HSP72. It is possible that differential tissue expressions of ERα and ERβ are responsible for the tissue-specific responses of HSP72 in females, and future studies will be needed to address this possibility.

The role of testosterone in the expression of HSP72 has also been studied, and another interpretation of how sex steroids affect HSP72 has been suggested. Testosterone has been shown to inhibit the HSP72 response (111), and it is plausible that it is the balance between estrogen and testosterone that is responsible for a normal induction of HSP72. The blunted HSP72 response following menopause, therefore, may be due to the decreased ratio of estrogen to testosterone (74).

Although this study has some limitations due to its descriptive nature, it adds a much needed characterization of the HSP72 response to acute stress in several peripheral tissues in both males and females. Our attempt to use the natural fluctuations of the estrous cycle to manipulate the HSP72 response offers insight into the adaptability of the estrogen system and its integration with cell systems. We believe that this profile of the HSP72 response will act as a springboard for future molecular and whole organism experiments concerning the interactions of sex steroids and HSP72.
The results of this study add to the current literature of sexual dimorphisms of the stress response. To our knowledge, this is the first study to compare the male and female intracel-
ular HSP72 response in many peripheral tissues and to char-
acterize the female HSP72 response across the estrous cycle. Male and female rats have unique HSP72 levels, depending on tissue type, which may be due to SNS innervation and differ-
ential estrogen effects across female peripheral tissues. Al-
though differences between HSP72 induction in males and females are large, the relatively minor fluctuations of sex hormones during the estrous cycle are not sufficient to alter HSP72 induction in female rats.

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