# Treadmill exercise training and estradiol increase plasma ACTH and prolactin after novel footshock

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**White-Welkley, Jill E., Gordon L. Warren, Bradford N. Bunnell, Edward H. Mougey, James L. Meyerhoff, and Rod K. Dishman.** Treadmill exercise training and estradiol increase plasma ACTH and prolactin after novel footshock. *J. Appl. Physiol.* 80(3): 931–939, 1996.—We examined whether rats that were treadmill exercise trained (Tr) or chronically immobilized (CI) had similar responses by the hypothalamic-pituitary-adrenal (HPA) cortical axis to acute stress and whether the HPA responses interacted with the hypothalamic-pituitary-gonadal (HPG) axis. After 6 wk (1 h/day, 6 days/wk) of Tr or CI, plasma concentrations of adrenocorticotropic hormone ([ACTH]), [prolactin], and [corticosterone] were measured after familiar (treadmill running or immobilization) or novel (footshock) stress. Ovariectomized Sprague-Dawley females ($n = 72$) were implanted with capsules containing estradiol benzoate ($E_2$) and randomly assigned in a 2-group ($E_2$ vs. no $E_2$) × 3 treatment (Tr vs. CI vs. sedentary) × 4 acute stressor [footshock vs. treadmill running (Run) vs. immobilization (Im) vs. no stress] × 3 recovery time (1 vs. 15 vs. 30 min) mixed-model analysis of variance. $E_2$ capsules were removed from one-half of the animals 48 h before the first stressor session. After 10 min of acute stress, blood was drawn from a jugular catheter at 1, 15, and 30 min of recovery. [ACTH] and [prolactin] after footshock were higher in Tr rats with $E_2$ compared with CI and sedentary rats without $E_2$; recovery levels for sedentary animals were higher after Run compared with Im. The elevation in [corticosterone] from *minute 1* to *15* of recovery was higher after the familiar Run and Im conditions. Our findings are consistent with an increased responsiveness of the HPA axis to novel footshock after treadmill exercise training that is additionally modulated by the HPG axis.

footshock; immobilization; hypothalamic-pituitary-adrenal cortical; hypothalamic-pituitary-gonadal; ovariectomized; adrenocorticotropic hormone

MOST STUDIES of physiological responses to stress after exercise training have been limited to cardiovascular and sympathetic-sympathoadrenal medullary responses (50). It is important to also understand adaptations of the hypothalamic-pituitary-adrenal (HPA) cortical axis after exercise training because they are involved in models of the pathogenesis of cardiovascular disease (32), anxiety disorders (21), major depression (18), and immunocompetence (5). Studies examining the question of whether exercise training alters the HPA axis usually have been conducted under conditions of exercise, rest, or pharmacological challenge and yielded mixed results (7, 8, 19, 26, 36, 37, 43, 54). Another approach to the question is to examine the effect of exercise training on the magnitude and recovery of physiological responses of the HPA axis elicited by standardized stressors other than exercise, thus testing the hypothesis that exercise training induces cross-stressor adaptations in HPA responsiveness (50). Studies using this approach also have yielded conflicting results (6, 14, 45, 55, 56), partly because the authors did not *1*) compare acute responses to exercise with responses to other stressors that are familiar (i.e., homotypic) vs. novel (i.e., heterotypic) (50) or *2*) consider influences on exercise responses exerted by reproductive hormones known to influence physiological responses to nonexercise stressors.

Many types of acute stress, including exercise (28), stimulate the release of HPA hormones (29, 30). However, physiological adaptations in HPA responses to heterotypic stress after exercise training have received little study (6, 14, 45, 55, 56). After exercise training, exercise may be referred to as a familiar or homotypic stressor. Conversely, heterotypic stress denotes any psychophysiological condition to which an organism has had no prior experience. One cross-sectional study in humans reported no effect of physical fitness on plasma adrenocorticotropic hormone (ACTH) levels after a familiar psychomotor task (6), but it is not known whether HPA responses are attenuated or sensitized to heterotypic stressors after exercise training (50, 52). Exercise studies in this area also generally failed to consider the interaction of stress responses with extra-HPA neuroendocrine mechanisms, despite evidence of such an interaction in highly trained human females (36). Hormones of the hypothalamic-pituitary-gonadal (HPG) axis alter HPA responses to stress. In particular, estrogen increases plasma levels of ACTH and corticosterone after restraint or immobilization and acute running (53, 56). Also, the presence of estrogen appears to be critical for the stress-induced release of prolactin in female rats (56).

In the present experiment, changes in the plasma concentrations of HPA hormones, i.e., ACTH, corticosterone, and prolactin, were examined in rats that were treadmill exercise trained, chronically immobilized, or sedentary and then exposed to acute bouts of homotypic (running or immobilization) or heterotypic (footshock) stress or no stress. Chronic immobilization was employed to determine whether HPA adaptations to exercise treadmill training were unique in comparison with an established intermittent stress protocol (23, 28) known to elicit acute and chronic changes in HPA hormone levels (28). The duration of immobilization can be adjusted to correspond to repeated exercise exposure, and immobilization provides a suitable paradox to the locomotory stimulus of exercise. We previ-

ously reported (56) that HPA responsiveness to acute running and immobilization after treadmill exercise training was increased by acute (3 days) estradiol injections. However, the activation effects of estradiol on neurochemical changes in the brain may be related to the dose and duration of estradiol exposure (38). Hence, in the present experiment, we used chronic estrogen replacement after ovariectomy.

A 6-wk treadmill exercise training protocol at ~50–60% peak $O_2$ uptake ($\dot{V}O_{2peak}$) (4) was used to induce an exercise training effect indicated by enzymatic assay of succinate dehydrogenase (SDH) activity in soleus locomotory muscle. Treadmill running appears to be a preferred exercise training protocol in the study of HPA adaptations to exercise (56) compared with voluntary wheel running. We previously found that chronic wheel running did not alter plasma levels of ACTH, corticosterone, or prolactin after footshock (16). Serial sampling of plasma levels of the HPA hormones during recovery from acute stress advances our previous work (56) to better determine the patterns of recovery by HPA hormones in exercise-trained animals.

## METHODS

Ninety female Sprague-Dawley derived rats weighing ~80 g were purchased from Charles River. Before experimental manipulations, animals were allowed to adapt to the vivarium for 1 wk. The rooms in which they were housed were maintained at 23 ± 2°C. Food and water were available ad libitum. A constant light-dark cycle was maintained with lights on at 0600 and off at 1800.

A 3 × 2 × 4 × 3 mixed factorial experimental design with factors 3 and 4 repeated was used (Table 1). The independent variables were *1*) treatment condition: chronic daily stress (treadmill running or immobilization) or sedentary; *2*) group: the presence or absence of the estrogen-containing Silastic capsules during all acute-stress sessions; *3*) stressor: heterotypic (footshock), homotypic (acute treadmill running or immobilization), and no-stress conditions; and *4*) time: *minutes 1, 15*, and *30* of recovery following acute stress. The sedentary-treatment animals were further divided randomly into two control groups: *1*) sedentary (acute running) and *2*) sedentary (acute immobilization) to compare the effects of acute running or immobilization between sedentary control animals and the chronically immobilized or treadmill-trained animals. The dependent measures were plasma concentrations of ACTH, corticosterone, and prolactin detected by radioimmunoassay (RIA).

Table 1. *Experimental design*

| Treatment Condition | Group | Acute Stressor Condition (Counterbalanced) |
|---|---|---|
| Treadmill exercise training | Estradiol capsule intact $n=9$<br>Estradiol capsule removed $n=9$ | Run<br>Footshock<br>No stress |
| Chronic immobilization | Estradiol capsule intact $n=8$<br>Estradiol capsule removed $n=9$ | Immobilized<br>Footshock<br>No stress |
| Sedentary | Estradiol capsule intact $n=16$<br>Estradiol capsule removed $n=16$ | Run<br>Immobilized<br>Footshock<br>No stress |

In each condition, $n$ = no. of rats that completed the experiment. In sedentary treatment conditions, one-half ($n=8$) animals ran and one-half ($n=8$) were immobilized during acute-stressor conditions.

## EXPERIMENTAL PROCEDURES

After 1 wk of adaptation to the vivarium, when the animal's weight was stable and age approximated 40 days, bilateral ovariectomy surgery was performed on all animals under aseptic conditions. Capsules containing estradiol benzoate ($E_2$) were prepared from Silastic medical tubing 0.058 in. ID, 10 mm long (Dow Corning), and filled with 8.5 μl of 2% estradiol in cholesterol (Sigma Chemical, St. Louis, MO). Estradiol capsules were soaked in 0.9% saline for 24 h before implantation to facilitate initial hormonal surge. Capsules were implanted into a small slit in the skin on the back of the neck (2). Estradiol capsules remained in all animals during the 6 wk of treadmill exercise training and chronic immobilization to control for the possible chronic influence of estrogen on HPA adaptations. The chronic $E_2$ implantation procedure before the experimental treatment was used to extend our previous study employing an acute 3-day $E_2$ injection protocol after exercise treadmill training that led to increased plasma ACTH after acute immobilization stress (56). After the ovariectomy surgery, the animals were allowed 48 h of recovery time. All animals were similarly weighed and handled daily for the duration of the experiment. Body mass increased equally for all treatments from *wk 1* (mean ± SD; 114 ± 18 g) through *wk 8* (274 ± 128 g). No treatment or treatment-by-time effects were found for body mass, $F(2, 24) = 1.43$, $P = 0.36$.

*Treadmill exercise training and chronic immobilization.* After recovery from ovariectomy surgery, animals were familiarized with running on a Stanhope 2000 (Davis, CA) motor-driven treadmill (5 min, 15 m/min at 0° incline) during a 3-day period. On each of the 3 days, running performance was rated on a scale of 1 to 5, with 5 being the highest rating (17). At the end of the trial period, the animals receiving a mean rating of 3 or greater ($n = 72$) were randomly assigned to the experimental conditions. In our experience with this treadmill accomodation protocol, ~80–90% of Sprague-Dawley rats learn and are motivated to perform well (a rating of 3 of greater) (17). Poor runners were excluded from the study to minimize dropouts during training and to optimize the group exercise training effect. We have previously reported that, when using the Sprague-Dawley strain, treadmill running performance during the accomodation period is unrelated to a behavioral measure of stress (17) or to plasma ACTH, prolactin, or corticosterone levels after acute immobilization stress (56). Hence, it is unlikely that the exclusion of poor runners from the present experiment reduced the generalizability of HPA responses. Animals assigned to the treadmill exercise training condition adapted to 0° incline treadmill running over a 2-wk period. The adaptation period consisted of gradually increasing the time from 15 to 30 min and the speed from 15 to 25 m/min during the first week. At the end of the second week, animals ran for 60 min at 30 m/min. This protocol was maintained during the light phase (1000–1400) for 6 consecutive wk. When the equation for level treadmill running of Armstrong and colleagues (4) is used, this speed elicits an estimated $O_2$ uptake of 68 $ml \cdot kg^{-1} \cdot min^{-1}$, or

~50–60% $\dot{V}O_{2peak}$ for the rat. It produces a significant increase in oxidative capacity, as measured by SDH activity, in slow-twitch oxidative and fast-twitch oxidative glycolytic muscle fibers (3). Chronically immobilized animals were restrained in ventilated mylar cones at the same time of day and for the same duration as the treadmill-trained rats.

*Catheter procedure*. After 6 wk of treadmill exercise training and chronic immobilization, chronic catheters were implanted in the right jugular vein (24) under methoxyflurane (Metafane; Pitman-Moore, Washington Crossing, NJ) anesthesia. The Silastic catheter tubing was run subcutaneously to the back of the neck where it was externalized and protected by a two-sided Velcro patch. The catheters were flushed daily with dilute heparinized saline (200 units/ml) and filled with a heparinized glycerin solution (1,000 μl/ml) before the protective Velcro patch was replaced. Estradiol capsules were removed from one-half the animals in all experimental conditions during the catheter procedure to examine more directly the possible acute effect of estradiol on HPA responses after treadmill exercise training. As expected, animals with estradiol capsules had significantly higher plasma estradiol concentration ([estradiol]) (24.85 ± 8.2 pg/ml) compared with the animals without capsules (7.56 ± 3.87 pg/ml), $t(12) = 1.9$, $P < 0.05$, at the time of catheter surgery, before the first acute-stress session. The general condition of the animal was evaluated at this time. A 48-h recovery period was allowed for plasma [estradiol] to drop in the animals in which the estradiol capsules had been removed and to allow elevated postsurgery neuroendocrine values to return to baseline before the first acute-stress session. The treadmill-trained and chronically immobilized animals maintained their normal exercise or immobilization schedule when possible during the recovery days, but not all animals tolerated the treadmill running on their recovery days, and for them both the duration and speed were reduced. We were unable to obtain blood withdrawals for 5 of the 17 treadmill-trained animals on the first day of acute stress. These animals were replaced by an additional group of six treadmill-trained animals (three $E_2$ and three no $E_2$) of the same age and mass that underwent the treadmill exercise training protocol.

*Acute-stress sessions*. After the 48-h period of recovery from catherization surgery, the animals were exposed to the first of three counterbalanced acute-stress sessions: *1*) heterotypic (footshock), *2*) homotypic (running or immobilization), or *3*) no-stress conditions. All acute-stress sessions occurred between 1000 and 1400 to minimize circadian effects. The second and third stress sessions were 48 and 96 h, respectively, after the first session. The acute-stress (immobilization and treadmill running) sessions were conducted as previously described for a 10-min duration. Scrambled, uncontrollable footshock was delivered for 15 s every minute during a 10-min period by a BRS Foringer Constant Power Shocker/Scrambler (model 3750) at a 1-mA intensity.

*Blood collection and preparation*. After each acute-stress session, the animal was transported to the vivarium and placed in its home cage. Extensions of the catheter tubing were attached into the original catheter to permit movement by the animal and to ensure minimal further disturbance to the animal during blood withdrawals. The average duration of time needed to transport the animal to its home cage and attach the catheter extension was 1–2 min, which is referred to as *minute 1* of recovery in the text and Figs. 1–4. The lost blood volume was replaced with 0.9% saline immediately after each withdrawal. Blood samples (0.5–0.6 ml) were collected using heparinized 1-ml plastic syringes at the following times: immediately following the acute stress (*minute 1* of recovery), *minute 15* of recovery, and *minute 30* of recovery. Samples were transferred to 1.5-ml Eppendorf tubes and spun in a microfuge at 2,000 *g* for 5 min at 4°C. Plasma was removed and transferred into 1.5-ml Eppendorf tubes containing 20 μg of aprotinin. The samples were stored on ice for ~1 h until frozen and stored at −80°C. Microcapillary tubes were filled from a portion of the blood samples drawn from the indwelling catheters, centrifuged, and analyzed for hematocrit. Hematocrit concentration was decreased at each of the first three blood draws on *day 1* and was stable thereafter. All hormone concentration values were corrected for dilution by heparin and aprotinin but were not corrected for the possible plasma volume shifts that may have occurred during the acute stressor sessions. The differences in hormone concentration levels between control and acute running conditions that we report well exceed those explainable by plasma volume shifts due to acute running (25). Plasma volume shifts that accompany acute exercise (hemoconcentration) can be attenuated after exercise training (25). Thus plasma hormone concentration differences in the treadmill-trained animals compared with the sedentary animals after the session of acute running may have been greater than we report.

Animals in which the catheter remained patent through the third acute-stress bout were immediately decapitated after the last blood withdrawal. Animals in which catheters failed to remain patent at any time during the expermental procedure (~10% in each experimental condition) were immediately decapitated at *minute 1* of recovery on the next stress day. The soleus muscle was removed, frozen in liquid nitrogen, and stored at −80°C until SDH activities were determined to assess the effect of treadmill exercise training on oxidative muscle enzyme activity (12).

*Estradiol sampling procedure*. During catheter surgery, the Silastic estradiol capsules were removed from one-half of the animals randomly selected from the experimental conditions. To compare [estradiol] in the with-capsule vs. without-capsule groups, samples were taken from select ($n = 14$) animals during jugular catheterization and *minute 1* of recovery on *days 1, 2*, and *3* of the acute-stress sessions. All experimental conditions were represented. Plasma from these samples was analyzed using a RIA kit purchased from Diagnostic Systems Laboratories, Webster, TX (catalog DSL 4400). Estradiol concentrations <8 pg/ml were considered negligible.

*Blood and muscle assays*. Pituitary-adrenal cortical hormones were assayed by RIA. RIA for corticosterone was performed as described previously (40). The sensitivity of the assay was 2.0 μg/100 ml plasma. The intra- and interassay coefficients of variation were 5 and 10%, respectively. Materials for the RIA of prolactin were provided by the National Institutes of Health through the Rat Pituitary Hormone Distribution Program. Sensitivity for the prolactin assay was ~0.8 ng/ml. The intra- and interassay coefficients of variation were 6 and 12%. The procedure for the ACTH assay using a commercial kit (Incstar, Stillwater, MI, catalog no. 24310) has been described previously (41). Assay sensitivity was 5.0 pg/ml. The within-assay coefficient of variation was 8.2% at 33.0 pg/ml and 2.0% at 112 pg/ml. The interassay coefficient of variation was 10.6% at 33.0 pg/ml and 6.4% at 109.0 pg/ml. SDH activities were measured after homogenization in a 33 mM phosphate buffer (pH 7.4) by following cytochrome *c* reduction at 550 nmol and 25°C (12).

*Statistical analysis*. Statistical procedures employed PC SAS version 6.04 (SAS Institute, Cary, NC). Outliers were determined using Grubb's test statistic at an alpha level of 0.05. Missing data were replaced with cell means when more than five of the nine possible samples were available; replaced data approximated 10% of all observations. Cell sizes

ranged from eight to nine animals. The General Linear Model type III error term was used to account for unequal cell sizes. Degrees of freedom were adjusted by the method of Greenhouse-Geisser when the sphericity assumption was violated for the repeated factors of stress and time. An alpha level of $P < 0.05$ was considered statistically significant. Sample size was estimated by a power procedure based on a large effect size (omega squared ~0.20) averaged from protocols similar to ours. Nine animals per cell for the between-subject and within-subject factors yielded a power of ~0.70 at an alpha of $P < 0.05$. Values are presented as means ± SD in the text and means ± SE in Figs. 1–4.

## RESULTS

Plasma concentrations of ACTH ([ACTH]) and prolactin ([prolactin]) after heterotypic footshock were greater in treadmill-trained animals with estradiol supplementation when compared with animals without estradiol that were chronically immobilized or were sedentary. No group (estradiol vs. no estradiol) main effect was found for corticosterone, ACTH, or prolactin. ACTH levels in the sedentary animals after acute running were greater than those observed after acute immobilization or footshock.

*Corticosterone*. The analysis of variance (ANOVA) for corticosterone concentration ([corticosterone]) during recovery indicated a significant acute-stressor effect, $F(2,78) = 26.99$, $P < 0.0001$ (Fig. 1). Post hoc contrasts revealed that corticosterone levels were lower under the no-stress, control condition compared with heterotypic footshock and homotypic stress (run or immobilization). Levels for both corticosterone and ACTH during the no-stress condition were elevated in comparison to established control values (28), due apparently to unavoidable stress associated with the serial catheter sampling. Because these elevations were uniform across experimental groups, they did not affect the experimental treatment and/or group comparisons. There was no main effect for group (estrogen), $F(1,78) = 0.84$, $P = 0.36$. However, there was a significant stressor-by-time interaction, $F(4,156) = 2.81$, $P < 0.03$. Corticosterone levels in the homotypic condition increased more from *minute 1* of recovery to *minute 15* of recovery, compared with the heterotypic and no-stress conditions.

*ACTH*. The ANOVA for [ACTH] indicated a significant stressor effect, $F(2,76) = 30.48$, $P < 0.0001$ (Fig. 2). Post hoc contrasts revealed that [ACTH] was lower in the no-stress condition compared with heterotypic footshock and homotypic stress (run or immobilization).

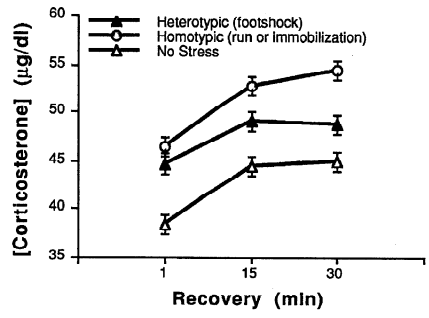


Fig. 1. Plasma corticosterone concn levels (concn shown as brackets) during recovery after heterotypic (footshock), homotypic (running or immobilization), and no-stress conditions. Values are means ± SE. There were no effects for group or treatment.

The stressor conditions elicited increases in [ACTH] from baseline control levels. There was no main effect for time or group. However, there was a significant stressor-by-time-by-treatment effect, $F(12,152) = 3.62$, $P < 0.0005$. ACTH levels were elevated in the sedentary animals after novel running at *minutes 1* and *15* of recovery, compared with the sedentary animals exposed to novel immobilization and to the animals in the no-stress condition. [ACTH] also was elevated at *minute 1* of recovery for the treadmill-trained animals after heterotypic footshock compared with the sedentary animals. [ACTH] was not elevated for the chronically immobilized animals after heterotypic footshock compared with the sedentary animals.

ACTH responses for the treadmill-trained animals to heterotypic footshock and homotypic running did not differ, nor did the [ACTH] differ between the chronically immobilized animals and the treadmill-trained animals. ANOVA indicated a stressor-by-time-by-group effect, $F(4,152) = 2.86$, $P < 0.03$.

Post hoc contrasts revealed [ACTH] was attenuated at *minute 1* of recovery after heterotypic footshock in the estradiol group compared with the no-estradiol group, but the groups did not differ at *minutes 15* and *30* of recovery (Fig. 3).

*Prolactin*. The ANOVA for [prolactin] indicated a significant stressor effect, $F(2,86) = 3.10$, $P < 0.05$. [Prolactin] values were lower after the no-stress condition compared with recovery after heterotypic footshock and homotypic (run or immobilization) stress (Fig. 4). There also was a stressor-by-treatment-by-group effect, $F(6,86) = 3.60$, $P < 0.01$.

Post hoc contrasts revealed that [prolactin] was elevated in the treadmill-trained animals with estradiol capsules after heterotypic footshock, compared with both the chronically immobilized and sedentary (acute immobilization) animals with estradiol. However, in the absence of estradiol, the treadmill-trained animal's prolactin response did not differ from the chronically immobilized or sedentary animals. In addition, prolactin levels were attenuated in the treadmill-trained and chronically immobilized treatment animals without estradiol capsules after homotypic stress compared with sedentary animals. However, prolactin responses did not differ by treatment after acute running and immobilization in animals with estradiol capsules (Fig. 4).

*SDH activity*. SDH activities in the soleus muscles of the treadmill-trained animals (1.42 ± 0.14 µmol cytochrome *c* reduced·min$^{-1}$·g wet wt$^{-1}$) were significantly greater [$t(2,50) = 2.15$, $P < 0.04$] than in the sedentary animals (1.04 ± 0.102 µmol cytochrome *c* reduced·min$^{-1}$·g wet wt$^{-1}$).

*Estradiol*. Before the first day of stress, animals with estradiol capsules had a significantly higher plasma [estradiol] (24.85 ± 8.2 pg/ml) compared with the animals without capsules [7.56 ± 3.87 pg/ml; $t(12) = 1.9$, $P < 0.05$]. [Estradiol] values in animals with

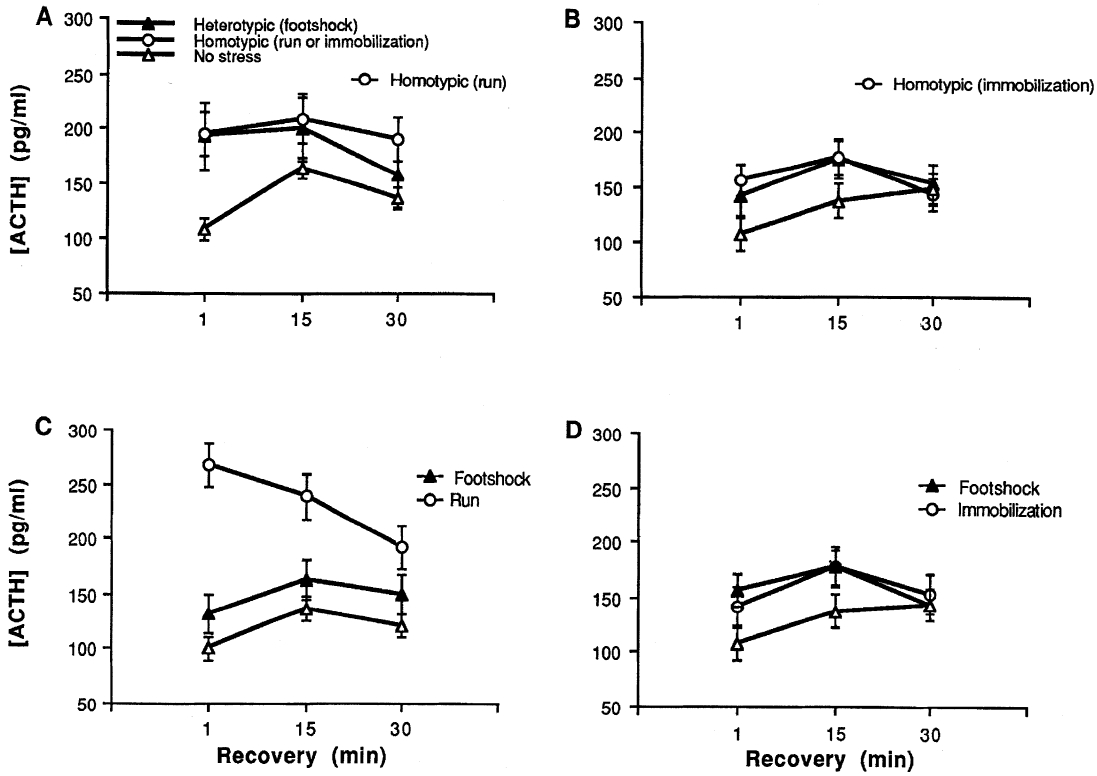


Fig. 2. Plasma adrenocorticotropic hormone (ACTH) concn levels in treadmill-trained (*A*), chronic immobilization (*B*), sedentary (acute running) (*C*), and sedentary (acute immobilization) (*D*) treatments during recovery after heterotypic (footshock), homotypic (running or immobilization), and no-stress conditions. Values are means ± SE. Stressor and stressor × time × treatment effects are shown. Levels in sedentary animals after heterotypic running were elevated at *minutes 1* and *15* compared with their levels after heterotypic immobilization.

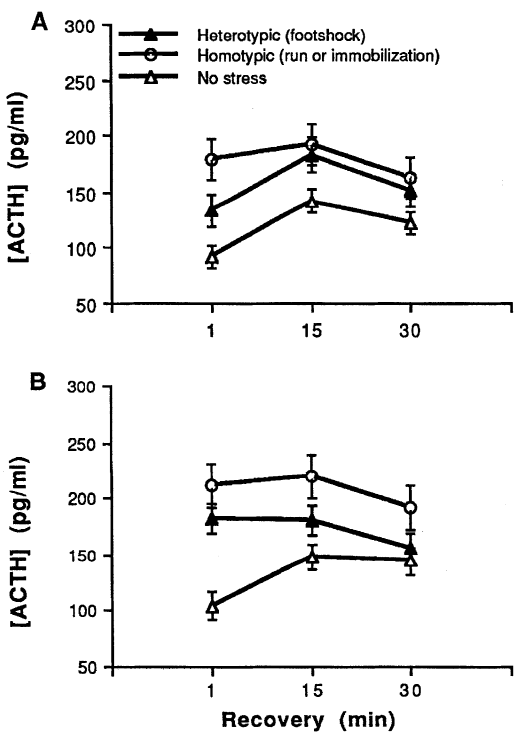


Fig. 3. Plasma ACTH concn levels in estradiol (*A*) and no-estradiol (*B*) groups during recovery after heterotypic (footshock), homotypic (run or immobilization), and no-stress conditions. Values are means ± SE. Stressor × time × treatment effect is shown. Levels at *minute 1* were attenuated after heterotypic footshock in estradiol, compared with no-estradiol, animals.

capsules were comparable to levels reported elsewhere employing similar implant methods (1).

## DISCUSSION

Plasma levels of HPA hormones after acute stress in ovariectomized female rats with and without estradiol capsules were studied under three training conditions and exposure to two different stressors. Repeated bouts of treadmill running, sufficient to elicit a training adaptation in locomotory muscle, resulted in greater responsiveness to heterotypic footshock. Plasma [ACTH] and [prolactin] after heterotypic footshock were greater in treadmill-trained animals with estradiol compared with animals that were chronically immobilized or compared with sedentary animals. Chronic immobilization stress did not elicit this effect. A greater increase in plasma corticosterone from *minute 1* to *minute 15* of recovery occurred after the acute homotypic stressors, but the overall pattern of recovery did not differ among the stressor or group conditions. ACTH levels in sedentary animals after acute treadmill running were greater than those observed after acute immobilization or footshock, suggesting that forced treadmill running is a more potent stressor of the HPA cortical axis than is immobilization or footshock.

The increased responsiveness of ACTH in the treadmill-trained animals is in contrast to results from Wantanabe and collegues (55), who reported an attenuated ACTH response in male rats after cage-switch

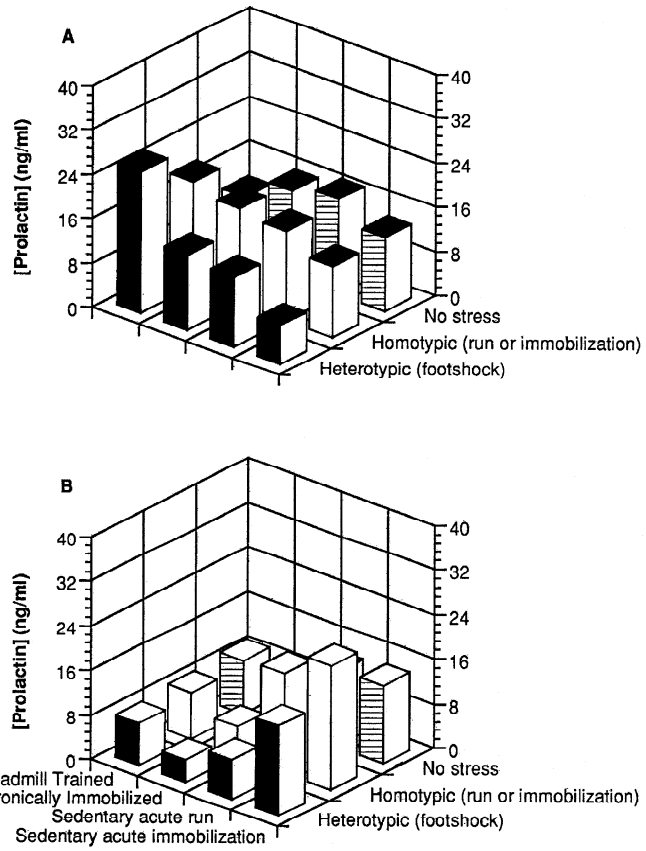


Fig. 4. Plasma prolactin concn in estradiol (*A*) and no-estradiol (*B*) groups during recovery after heterotypic (footshock) (solid bars), homotypic (running or immobilization) (open bars), and no-stress conditions (hatched bars). Values are means ± SE. Stressor × treatment × group effect is shown. Levels were elevated in treadmill-trained animals with estradiol after heterotypic footshock compared with chronically immobilized and sedentary animals after acute immobilization with estradiol. Levels in chronically immobilized and treadmill-trained animals without estradiol were attenuated after homotypic stress compared with sedentary animals.

stress that followed repeated swim or treadmill exercise stress. Differences in strain and gender and the greater intensity of footshock stress compared with cage-switch stress preclude direct comparisons of that report with our results. Our observation of no attenuation of corticosterone levels after the heterotypic footshock or the homotypic run conditions in the exercise-trained animals is consistent with previous observations in rats (14, 56). Conversely, attenuations in corticosterone and cortisol have been reported after homotypic exercise stress (36, 55). An adaptation of attenuated corticosterone responses after exercise training is difficult to demonstrate because plasma corticosterone reaches near-maximum levels after relatively mild stressors.

Acute stress increases the release of ACTH and prolactin from the pituitary gland and the release of corticosterone from the adrenal cortex in the rat (28, 30). In general, repeated exposure of rats to a homotypic stressor results in reduced (habituated) plasma levels of prolactin (28, 56) and ACTH (28) after subsequent exposure to the same stressor compared with control animals. Hyperresponsivness, or sensitization, of HPA hormonal responses has been observed after periods of repeated homotypic stress; these responses appear to be dependent on the duration, type, and intensity of the acute and repeated stress and on the sex of the subject (44). In humans, plasma prolactin (15), ACTH (19, 43), and cortisol (37) increase during exercise and have been shown to be dependent on duration and intensity (37, 43). Cardiorespiratory fitness does not appear to moderate plasma levels of ACTH at submaximal exercise of moderate-to-high intensity (19, 43), but highly fit men have elevated ACTH levels after supramaximal exertion compared with untrained men (19). Thus our findings extend the aforementioned results of HPA hyperresponsiveness to the homotypic stress of exercise after exercise training by showing increased ACTH levels in response to a heterotypic stressor after exercise training.

We did not find differences in the treadmill-trained animals compared with the sedentary animals in their rate of recovery toward baseline values. Similarly, the treadmill-trained animals did not demonstrate elevated plasma ACTH and prolactin levels during the no-stress condition compared with the sedentary animals, as has been demonstrated in some human exercise studies (8) that examined HPA cortical plasma hormone values following exercise training. Our present results agree with our earlier findings under similar conditions (56).

Prolactin responses of the treadmill-trained and chronically immobilized animals without estradiol capsules were attenuated after acute running or acute immobilization when compared with the sedentary animals. In contrast, the animals with estradiol capsules did not show attenuation to the homotypic stressors but demonstrated an increased responsiveness after heterotypic footshock. The blunted prolactin responses by treadmill-trained and chronically immobilized animals after acute heterotypic footshock in animals without estradiol could be due to a lowering of circulating estradiol below physiological levels. However, because their responses were less than those of sedentary animals without estradiol replacement, it appears more likely that the lowered estradiol after capsule removal resulted in a reduced capacity for prolactin release in the treadmill and chronically immobilized animals. Increased prolactin levels have been observed in female rats (51) and humans (7) after exercise training when estrous and menstrual status were described but not controlled. In both studies, the magnitude of exercise training was high (i.e., running 7 days/wk, 60 min/day, 20 m/min for 10 wk in the female rats and 15 mo of marathon training in the women). The intensity (50–60% $\dot{V}O_{2peak}$) and duration (60 min/day for 6 wk) of the treadmill exercise training we employed was moderate (4). It is not known whether lower intensity and shorter duration of exercise training will induce increased responsiveness in prolactin.

The mechanisms that might explain our observations are not fully established. Prolactin secretion is mainly under inhibitory hypothalamic control (5, 42). Dopamine (DA) released from the tuberoinfundibular dopaminergic neurons impinges on DA-2 receptors located on the lactotroph cells of the anterior pituitary that

regulate the secretion of prolactin (5). Other peptides participate in the stimulation of prolactin under different physiological conditions, including β-endorphin and Met-enkephalin, thyrotropin-releasing hormone, vasoactive intestinal polypeptide, oxytocin, and galinin (42). Thus it is unlikely that prolactin release is solely controlled by the withdrawal of DA's inhibitory tone. Estrogens have an antidopaminergic receptor-mediated effect on prolactin secretion (5, 42). We are unaware of exercise training studies examining DA activity in brain areas relevant to prolactin control. Thus conclusions regarding the influence of treadmill exercise training or increased fitness on DA activity or on possible releasing factors for prolactin await further study.

The regulation of ACTH secretion also is under multifactorial control (33). Corticotrophin-releasing hormone (CRH) is recognized as the most potent releasing factor, but the effect of exercise training on the regulation and activity of CRH is not established. Other hypothalamic secretagogues including vasopressin, oxytocin and catecholamines act directly on the pituitary to release ACTH and/or indirectly through their ability to synergize with CRH (39). CRH release is inhibited by norepinephrine and DA in vitro (1, 46). However, norepinephrine has been reported to inhibit, stimulate, and not influence ACTH secretion and adrenocortical activation in vivo (46). Serotonin (5-HT) appears to facilitate CRH release, whereas γ-aminobutyric acid inhibits CRH. Changes in the levels of norepinephrine, DA, and 5-HT in specific brain areas have been observed after both exercise training (10, 18) and estradiol replacement (47). In what way brain monoamine levels after exercise relate to the secretion of ACTH and prolactin is not known.

Arginine vasopressin (AVP) has stimulatory effects on ACTH secretion (39). An increase in ACTH secretion in fit male and female runners has been reported after supramaximal exercise, despite ovine CRH infusion designed to saturate corticotrophs (49). Similarly, Heuser and colleagues (26) found an increased cortisol response in runners at rest after dexamethasone treatment and CRH challenge, suggesting that factors other than, or in addition to, CRH may influence ACTH release. The investigators in both studies suggested AVP as a releasing factor for ACTH. In nonprimates, AVP in peripheral circulation increases during severe physical stress (hemorrhage, electric shock, hypoglycemia) but not during behavioral stress (novel environment, restraint) (22). Plasma AVP has been shown to increase after acute exercise at intensities >45% $\dot{V}O_{2peak}$ (11). The increases in AVP during exercise are similar to levels observed after surgery and fluid deprivation (22). Cross-sectional and prospective human studies have reported lower plasma levels of AVP after exercise in trained men compared with control subjects (11). Increased AVP appears related positively to exercise intensity and duration. Our treadmill-trained animals ran at 50–60% $\dot{V}O_{2peak}$, 1 h/day, 6 days/wk for 6 wk. This amount of exercise could induce AVP adaptations via plasma volume shifts or somatosensory afferents from exercising muscles. Acute increases in AVP might override the negative feedback of corticosterone that inhibits ACTH secretion at the hypothalamus and pituitary, thus permitting increased plasma concentrations of corticosterone to be maintained to preserve blood glucose via the lipolytic and gluconeogenic effects of glucocorticoids. An adaptation of ACTH releasing factors in addition to CRH during treadmill exercise training might account for the greater increase in plasma ACTH after heterotypic footshock in the treadmill-trained animals despite high levels of corticosterone.

Mounting evidence (1, 8, 9, 36, 53) supports the finding that the HPA and HPG axes interact in response to stress. In the present study, ACTH responses in the estradiol group were attenuated after acute stress when compared with the no-estradiol group. This finding is consistent with our previous work (56) but not with other findings (35). Burgess and Handa (9) used estradiol implants and reported increased plasma corticosterone and a prolonged recovery of ACTH and prolactin after footshock. These investigators suggested that estradiol can attenuate glucocorticoid receptor-mediated delayed or slow negative feedback. Other investigators have suggested that estradiol may act through type I glucocorticoid receptors at the anterior lobe of the pituitary (20). The attenuated ACTH response we observed in the estradiol group may have been influenced by the severity and/or type of stressor utilized. The stimulatory and inhibitory influences of estrogen on the synthesis and release of ACTH secretagogues (44) such as oxytocin and AVP appear to differ in response to stressors of varying types or intensities (22). Footshock and treadmill running lead to greater HPA responses than does immobilization (44).

Our finding of an attenuated ACTH response after acute stress in the animals with estradiol capsules is in contrast to previous reports in rats exhibiting plasma [estradiol] characteristic of proestrus levels (53) but is consistent with ACTH levels observed during estrus (53) and with our previous findings (56). There is a dose-dependent relationship between plasma estradiol concentrations and estrogen-receptor messenger RNA in the ventromedial, ventrolateral, and arcuate nuclei of the hypothalamus (34). Also, ACTH levels in response to stress are directly related to plasma [estradiol]. Rats in proestrus show significantly higher ACTH levels during stress than animals in estrus or diestrus (53). The absence of main effects of estradiol on hormone levels in our study may have been influenced by the experimental procedures. In a previous study (56), we replaced estradiol by intramuscular injections 48 h before acute immobilization and observed a main effect of estradiol on corticosterone levels compared with ovariectomized rats that did not receive estradiol replacement. In the present study, mean plasma [estradiol] in animals implanted with capsules containing $E_2$ (25 pg/ml) were significantly higher than in animals without $E_2$ capsules and were comparable to levels reported elsewhere under similar conditions (1). However, variations in the time course and dose of estradiol's action on the HPA may have influenced our results. Experiments utilizing shorter implant periods (9) with considerably higher [estradiol] at the time of stress

have demonstrated significant effects for ACTH and corticosterone. To ensure the maintenance of oxidative enzyme changes in the soleus muscle, we restricted the period between capsule removal and the first acute-stress session to 48 h. Estrogen effects on monamine content in brain nuclei that regulate pituitary responses have been observed 45 h after treatment (47).

Our failure to observe effects of repeated immoblization stress on ACTH and prolactin levels after heterotypic footshock is in contrast with a previous study (30). However, the total period of exposure may have influenced HPA response patterns. The 6-wk duration of immobilization we employed was longer than the durations of 1–2 wk typically used by previous investigators and could lead to a reversal of attenuated HPA responses occurring earlier. A shorter span of repeated stress and the use of a milder heterotypic stressor such as cage-switch stress (55) can determine whether HPA hormone responses are dependent on duration and/or intensity of the stressor.

Our finding of increased plasma [prolactin] and [ACTH] after heterotypic footshock in treadmill exercise-trained rats treated with estradiol is consistent with an increased HPA responsiveness that interacts with the HPG axis. This observation extends prior findings of elevated ACTH after homotypic supramaximal exercise in highly trained humans (19) to an exercise training-induced hyperresponsiveness of plasma ACTH and prolactin after exposure to a heterotypic stressor. It has been proposed that cross-stressor hyperresponsiveness after increased fitness is an adaptation with potentially positive consequences for homeostasis and health (13, 50). An increased or sensitized responsiveness by fit animals in hypothalamic pituitary variables after heterotypic stress can yield a heightened energy capacity that may prove beneficial in physiologically challenging situations. The dependence of the prolactin responses on the presence of estradiol may have important implications for further research seeking to understand the gender differences in HPA responses that have been reported in the literature (27, 31) and may be relevant for understanding women's health (36, 48). However, the importance for health or performance of the increased responsiveness that we have observed after treadmill exercise training is not clarified by our report. Replications of our study are needed that concomitantly assess the responses of other systems (e.g., immune or cardiovascular) or outcomes indicative of disease. Future studies should examine the effects of treadmill exercise training on the regulation of central releasing factors for prolactin and ACTH at the level of the hypothalamus or anterior pituitary.

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