Effect of estrogen supplementation on exercise thermoregulation in premenopausal women

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1Department of Physical Education, National Ping Tung Teacher's College, Ping Tung, Taiwan; Departments of Exercise Science and Obstetrics and Gynecology, University of Iowa, Iowa City, Iowa 52242; and 3Department of Internal Medicine, University of New Mexico, Albuquerque, New Mexico 87131

Chang, R.-T., G. P. Lambert, P. L. Moseley, F. K. Chapler, and C. V. Gisolfi. Effect of estrogen supplementation on exercise thermoregulation in premenopausal women. J. Appl. Physiol. 85(6): 2082–2088, 1998.—This study examined the effects of 3 days of estrogen supplementation (ES) on thermoregulation during exercise in premenopausal (20–39 yr) adult women during the follicular phase of the menstrual cycle. Subjects (11 control, 10 experimental) performed upright cycle ergometer exercise at 60% of maximal O2 consumption in a neutral environment (25°C, 30% relative humidity) for 20 min. Subjects were given placebo (P) or β-estradiol (2 mg/tablet, 3 tablets/day for 3 days). All experiments were conducted between 6:30 and 9:00 AM after ingestion of the last tablet. Heart rate, forearm blood flow (FBF), mean skin temperature, esophageal temperature (Tₑ), and forearm sweat rate were measured. Blood analysis for estrogen and progesterone reflected the follicular phase of the menstrual cycle. Maximal O2 consumption (37.1 ± 6.2 L/min in P vs. 38.4 ± 6.3 L/min in ES) and body weight-to-surface area ratio (35.58 ± 2.85 in P vs. 37.3 ± 2.7 in ES) were similar between groups. Synthesis of 70-kDa heat shock protein was not induced by 3 days of ES. Neither the threshold for sweating (36.97 ± 0.15°C in P vs. 36.90 ± 0.22°C in ES), the threshold for an increase in FBF (37.09 ± 0.22 in P vs. 37.17 ± 0.26°C in ES), nor the slope of sweat rate-Tₑ relationship (0.42 ± 0.16 in P vs. 0.41 ± 0.17 in ES), nor the FBF-Tₑ relationship (10.4 ± 4.4 in P vs. 9.61 ± 3.46 in ES) was altered (P > 0.05) by 3 days of ES. We conclude that 3 days of ES by young adult women in the follicular phase of their menstrual cycle have no effect on heat transfer to the skin, heat dissipation by evaporative cooling, or leukocyte synthesis of 70-kDa heat shock protein.

core body temperature; forearm blood flow; sweat rate; heat shock protein; skin temperature; exercise

SEVERAL STUDIES INDICATE that estrogen replacement therapy in postmenopausal women reduces not only the physiological symptoms of estrogen withdrawal, such as the occurrence of hot flashes (21), but also the incidence of cardiovascular disease (37). Interestingly, there is controversy over the thermal adaptive response of women, particularly in relation to the menstrual cycle. Whereas some studies show no effect of sex hormones on adaptation to heat (15,17,20), more recent data demonstrate that estrogen supplemen-
tation (ES) lowers the core temperature threshold for the increase in forearm blood flow (FBF) and the initiation of sweating in postmenopausal women exercising in a warm environment (39). Similarly, rats treated with estrogen regulate their core temperature at a lower level during heat exposure and show an increased evaporative water loss at all levels of core temperature and a reduced core temperature threshold for the onset of saliva spreading (1).

Previous studies have shown that ES decreases (9,16,39), has no effect on (10,22), or increases core temperature (2,3,24). Silva and Bouland (36) found that estradiol excited 26% of warm-sensitive neurons and inhibited 4% of cold-sensitive neurons in preoptic tissue slices. This suggests that the hypothermic effects of estradiol are due to the direct actions of estradiol on preoptic neurons. In contrast, Cagnacci et al. (3) demonstrated that ES significantly increased the hyperthermic role of endogenous opioid peptides in postmeno-
pausal women. However, the data of Czaja and Butera (6) indicate that the effects of estradiol on rectal temperature (Tₑ) may be related to changes in peripheral blood flow rather than to a shift in core tem-
perature.

At the cellular level, heat shock proteins (HSPs) are produced in response to heat stress and are the central component of acquired heat tolerance (26). HSPs are differentially regulated in men and women, and increases in 70-kDa HSP (HSP 70) and 90-kDa HSP (HSP 90) occur in ovariectomized female rats following ES (28). Our laboratory was the first to show that HSP 70 may be produced in humans exercising in the heat, and thus HSPs may be useful biomarkers of both thermal history and thermal tolerance (33).

Because of the important link between ES conferring improved thermoregulatory responses in postmeno-
pausal women and rats and ES increasing HSP 70 in ovariectomized rats, we sought to determine whether ES would alter thermoregulatory responses in premeno-
pausal women during exercise. Previous research indicates that an assay of HSP 70 by blood mononuclear cells may be a useful biomarker to evaluate the potential relationship between ES and thermoregulatory responses in premenopausal women during exercise (33).

The purposes of this study, therefore, were to determine whether short-term ES improves thermoregulatory responses in premenopausal women and whether HSP 70 can be used as a biomarker to assess these responses. Because ES has been shown to enhance HSP synthesis and improve thermoregulatory responses during exercise in the heat, we hypothesized that 1) short-term ES could improve thermoregulatory responses in exercising women and 2) HSP might serve as a convenient and rapidly assayed biomarker of thermal tolerance and therefore could be used to de-
velop therapeutic strategies to optimize temperature regulation in women.
METHODS

Subjects. Twenty-one women, aged 21–39 yr, volunteered to participate in this study. Subjects were nonsmokers and reported having recurrent, normal menstrual cycles. They refrained from oral contraceptive use for at least 2 mo before the experiments. Each volunteer was informed of the testing procedures and the risk factors associated with the experiments, and written consent to participate was obtained. Subjects were given a physical examination, and experimental procedures were approved in advance by the Human Subjects Committee at the University of Iowa.

To minimize the possible interaction of progesterone and to maximize differences in estradiol levels between ES and control subjects, this study was conducted with women in the early follicular phase of their menstrual cycle. In the follicular phase, estradiol levels average 40–80 pg/ml. A short-term ES protocol (2 mg estradiol 3 times/day for 3 days), developed for use to elevate estradiol levels and tested for safety in in vivo fertilization programs, was used to elevate estradiol levels >1,000 pg/ml.

Serum estradiol and progesterone concentrations were measured to reflect levels associated with the follicular phase of the menstrual cycle, specifically, a low level of estradiol and an extremely low level of progesterone, if any at all. These values were demonstrated in the preexperiment levels in all subjects. All estradiol levels were between 18 and 40 pg/ml, which is appropriate for the early part of the follicular phase. All progesterone levels were <1.5 ng/ml, which is also normal for a follicular-phase level. In addition, the women were menstruating during the experiments and showed a normal luteal elevation in basal body temperature measured rectally.

With the use of a double-blind design, subjects were randomly assigned to ES (n = 10) or placebo (n = 11) groups. Experiments were conducted between November and December to preclude heat acclimatization. Age, maximal O2 consumption (V̇O2max), and body weight-to-surface area ratio were not significantly different between groups (Table 1).

Estimation of V̇O2max and submaximal O2 consumption. Before the experiment, each subject's V̇O2max was determined by an incremental cycle ergometer (The Bike, Cybex, Ronkonkoma, NY) test. Subjects completed three 3-min workloads of 75, 100, and 125 W. After the last 3-min period, the workload was increased by 25 W each minute until volitional fatigue. O2 consumption (V̇O2) and CO2 production (V̇CO2) were analyzed continuously (Q-Plex I Metabolic System, Quinton Instruments, Seattle, WA), and heart rates (HRs) were measured every minute. A maximal test was defined by the achievement of at least two of the following three criteria: 1) a plateau or decrease in V̇O2 despite an increase in workload, 2) a respiratory exchange ratio (RER) ≥1.1, or 3) attainment of at least 90% of predicted maximum HR (5). The workload required to elicit 60% V̇O2max was determined from submaximal V̇O2 values.

Study design. Each experiment consisted of a 5-min rest period followed by 20 min of cycle exercise at 60% V̇O2max. Cycling was performed in an upright posture in an environmental chamber maintained at 25°C and 30% relative humidity (RH). Subjects ingested two tablets of estradiol (Estrace, 2 mg/tablet; Bristol-Meyers Squibb, Princeton, NJ) on day 1 and three tablets on days 2 and 3 of their menstrual cycle. A final tablet was ingested 1–2 h before the experiment. All experiments were carried out between 6:30 and 9:00 AM. On the first day of the subject's menstrual cycle, a blood sample was obtained to determine estradiol and progesterone concentrations and for analysis of leukocyte HSP 70. Additional blood samples for HSP 70 analysis were obtained before the experiment and 6 and 24 h after ingestion of the last tablet. Serum estradiol and progesterone concentrations were also measured on the second blood sample.

Experimental protocol. Subjects were instructed to fast overnight and to refrain from heavy exercise for 24 h before the experiment. On arrival at the laboratory, subjects ingested 6 ml water/kg body weight to ensure hydration. A blood sample was obtained for HSP 70 determination and for estradiol and progesterone measurement. Body height and weight were measured, and a HR monitor (Polar Vantage XL, Polar USA, Stamford, CT) was placed around the chest at the level of the xyphoid process. Subjects then entered the environmental chamber and sat upright on the cycle ergometer for ≥45 min, allowing for instrument setup and postural equilibration. An esophageal thermocouple was orally positioned at a depth equal to 25% of each subject's height (40) to measure esophageal temperature (TES). Copper-constantan thermocouples were placed on the upper arm, chest, thigh, and lower leg for skin temperature [mean skin temperature (Tsk)] measurements (29). For FBF measurements, a double-lateral cuff and two pressure cuffs were placed on the forearm, arm, and wrist, respectively, and a capsule was placed on the right forearm cm below the antecubital fossa for determination of the local sweating response by resistance hygrometry. Measurement. TES was monitored continuously with a Tes sensor (ESO-1, Physitemp Instruments, Clifton, NJ). Changes in TES were plotted by a direct writing oscillographic recorder (model 1508A Visicorder, Honeywell, Denver, CO). Because swallowing saliva may lower TES, subjects expectorated all saliva into a cup.

Tsk was calculated by a weighted sum of four different skin temperature measurements (29). Tsk was calculated as upper arm (0.3) + chest (0.3) + thigh (0.2) + lower leg (0.2). Skin temperatures were collected each minute by an on-line computer system (IBM AT personal computer, IBM, Armonk, NY).

Venous occlusion plethysmography was used to measure FBF on the left arm by using an air-filled latex plethysmographic cuff fitted for each subject (Plethysmography Products, Korsor, Denmark). During the experiment, FBF measurements were obtained with the forearm extended laterally away from the torso and supported above the venostatic level. The left hand was excluded from the circulation with a pneumatic cuff at the wrist inflated to 180–220 mmHg. A second cuff placed around the upper arm was inflated to 50 mmHg twice per minute (15 s on/off interval). FBF was computed from the slopes of plethysmographic curves.

Local sweat rate (SR) of the right forearm was continuously determined from a ventilated lithium chloride sensor within a 6,605-cm² capsule (12). Flow rate was adjusted to optimize sensitivity and to ensure adequate data range for the chart
recorder. Calibration of the hygrometer system involves determining the trace deflection from baseline for a given change in air flow through the humidification system. A regression equation is determined for the relationship between trace deflection and saturated airflow. SR was calculated from the regression line by converting rates of air flow through the humidification system into equivalent SR values.

The FBF-T_{es} and SR-T_{es} thresholds were defined as the point after which a continuous increase in FBF or SR occurred. The slope of the linear regression relating the rise in FBF and SR to T_{es} was used to characterize the sensitivity of the skin blood flow and SR responses. Calibration procedures for FBF, T_{es}, T_{ski}, and SR measurements were conducted immediately before each experiment.

HSP analysis. Human leukocytes were isolated at room temperature (22°C) by adding 5 ml of Histopaque (Sigma Chemical, St. Louis, MO) to the blood (10 ml) and were centrifuged (3,000 rpm, 25–30 min). Leukocytes were collected and washed (gentle aspiration and centrifugation at 1,500 rpm for 5–7 min) twice with PBS. The final leukocyte pellet was suspended in 0.25 ml PBS and stored at -70°C until analysis. This isolation procedure was initiated immediately on attainment of a blood sample. HSP 70 analysis was performed as previously described by using Western blot analysis (33). Serum progesterone and estradiol concentrations were measured by using a radioimmunoassay (14).

Statistical analysis. Although a power test revealed that six subjects would yield 90% power, we opted to study four additional women for a total of 10 subjects. One- and two-factor ANOVAs were utilized with repeated measures as necessary to determine whether significant differences existed between groups and across time. Values presented are means ± SE. Linear-regression analysis was employed for each experiment to obtain thresholds and slopes of the SR vs. T_{es} and FBF vs. T_{es} relationships. A post hoc power analysis was conducted by using actual values obtained from this study to verify acceptance of the null hypothesis.

RESULTS

Serum estradiol concentration was unaffected by placebo treatment but was significantly elevated (P < 0.05) after ES (Table 2). Serum estradiol concentrations were similar (P > 0.05) between the two groups before ES and after placebo treatment. Serum progesterone concentration was not significantly different between the two groups or between pre- and post-ES (P > 0.05).

HR, T_{ski}, and T_{es} responses during exercise were not significantly different between ES and placebo groups (Fig. 1). Preexercise T_{es} values were 36.80 ± 0.06 and 36.81 ± 0.01°C for the ES and placebo groups, respectively. T_{ski} was lower in the ES group than in the placebo group, but the difference was not significant. Both groups showed a fall in T_{ski} at the onset of exercise and then an increase as T_{es} rose. The rate of rise in T_{ski}, T_{es}, and HR during exercise was not affected by ES.

FBF and SR responses during exercise were not significantly different between groups (Fig. 2). T_{es} threshold for SR, increase in FBF, and slope of T_{es}-SR and T_{es}-FBF relationships were plotted, with each line representing the data on a representative subject (Fig. 3, A and B). There were no significant differences between groups (Table 3).

Table 2. Serum estradiol and progesterone concentration

<table>
<thead>
<tr>
<th></th>
<th>Preplacebo</th>
<th>Postplacebo</th>
<th>Pre-ES</th>
<th>Post-ES</th>
<th>Early Follicular Phase (Normal Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol, pg/ml</td>
<td>34.8 ± 2.4</td>
<td>42.5 ± 4.2</td>
<td>32.55 ± 3.6</td>
<td>1,166.6 ± 114*</td>
<td>20–60</td>
</tr>
<tr>
<td>Progesterone, ng/ml</td>
<td>0.86 ± 0.07</td>
<td>1.02 ± 0.12</td>
<td>0.98 ± 0.11</td>
<td>0.99 ± 0.14</td>
<td>0.1–1.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. Preplacebo and postplacebo, before and after placebo treatment; pre-ES and post-ES, before and after estrogen supplementation. *Significantly different from pre-ES and pre- and postplacebo trials, P < 0.05.
Estradiol supplementation had no significant effect on blood mononuclear cell accumulation of HSP 70 after exercise. Figure 4 shows the HSP 70 accumulation in placebo (A) or ES (B) subjects before and 2 h after exercise. The post hoc power analysis using the HSP 70 values obtained from the present investigation revealed that 80 subjects would be necessary to achieve 80% power. Thus the null hypothesis was accepted.

**DISCUSSION**

The primary contribution of this study is that it provides evidence indicating that 3 days of ES during the early follicular phase of the menstrual cycle of premenopausal female subjects had no effect on thermoregulatory responses during exercise at 60% $\dot{V}O_2_{max}$ in a neutral environment ($25^\circ$C, 30% RH). $T_{es}$ thresholds for the initiation of sweating and increase in FBF were not altered by ES. $T_{es}$ also was not significantly different between groups during rest or after 20 min of exercise. SR, $T_{sk}$, HR, and FBF were similar between the two groups throughout exercise. Furthermore, HSP 70 synthesis was not induced by 3 days of ES.

Effect of ES on core body temperature. Previous studies have shown that ES decreases (9, 16, 39), has no effect on (10, 22), or increases core body temperature (2, 3, 24). These divergent findings are attributed, in part, to differences in the method of ES. However, the findings of this study suggest that the method of ES used in this study did not affect core body temperature.

**Table 3.** Slope and $T_{es}$ threshold of FBF and SR responses

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$T_{es}$ Threshold, $^\circ$C</th>
<th>Slope</th>
<th>$T_{es}$ Threshold, $^\circ$C</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen supplementation</td>
<td>37.17 ± 0.08</td>
<td>9.61 ± 1.10</td>
<td>36.91 ± 0.05</td>
<td>0.41 ± 0.05</td>
</tr>
<tr>
<td>Placebo</td>
<td>37.09 ± 0.08</td>
<td>10.04 ± 1.33</td>
<td>36.90 ± 0.07</td>
<td>0.42 ± 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. $T_{es}$, esophageal temperature; FBF, forearm blood flow; SR, sweat rate. Slope is expressed in ml·100 ml tissue$^{-1}$·min$^{-1}$·$^\circ$C$^{-1}$ and in mg·cm$^{-2}$·min$^{-1}$·$^\circ$C$^{-1}$ for FBF and SR, respectively.
part, to the following factors. 1) Differences in subject populations and/or species: Premenopausal, postmenopausal, and ovariectomized animals have served as experimental subjects in the above studies. 2) Phase of the menstrual cycle: It has been demonstrated (8, 13) that body temperature is regulated at a higher level during the luteal than the follicular phase of the menstrual cycle in women with normal periods; therefore, it is critical to control for both the time of day experiments are performed and the phase of the menstrual cycle, along with factors such as aerobic power, age, and body composition, when studying thermoregulatory responses to exercise in women (38). 3) Dosage of ES: Israel and Schneller (16) injected subjects with 1.5 mg estradiol compared with 18 mg given orally over 3 days in the present study or over 14–23 days in the study by Tankersley et al. (39). 4) Type of ES: It is not known what effect differences in estrogen formulations have on blood vessels (35). 5) Previous studies have not been conducted in similar environmental conditions. Based on the above, the effects of ES on temperature regulation in women warrant further investigation.

Effect of ES on FBF. In the present study, ES had no effect on FBF among premenopausal women (Fig. 2). However, previous studies suggest that acute (within 60 min) ES elevates blood flow in several vascular beds in both postmenopausal women and ovariectomized animals (11, 30, 41, 44, 45). The mechanism likely involves the production of nitric oxide from endothelial cells (4). In the present study, 3 days of ES is a longer term therapy relative to other acute ES studies (within 60 min). Long-term ES acts through a different cellular mechanism and includes regulation of gene expression, which opposes the acute potentiation of endothelium-dependent vasodilation (11). Thus it is possible that acute (within 60 min) administration could increase vasodilation in premenopausal women. However, evidence suggests that blood vessel integrity is well protected in premenopausal women, and estrogen may 1) inhibit the release of a vasoconstrictor substance such as endothelin (25), 2) decrease lipoprotein-induced smooth-muscle proliferation (7) in blood vessels, and 3) inhibit intimal proliferation associated with mechanical injury to endothelium (31, 32, 42). Thus relatively longer term ES had no effect on FBF in our subjects. This is likely due to the effects of longer term ES acting through a different mechanism compared with acute ES. It is also possible that blood vessel integrity is already well protected in the premenopausal woman.

Effect of ES on SR and skin temperature. In the present study, the slope of the $T_{es}-SR$ relationship was nearly identical in the ES and placebo groups. This agrees with the work of other investigators (8). In normal menstruating women without ES, however, Kawahata (18) demonstrated that there were rather large changes in the latent period of thermal sweating (time required to start sweating) measured at different times during the menstrual cycle. At the time of menstruation, the latent period was much shorter than the time of ovulation, and estrogen administration lengthened the latent period of sweating in men (18). Sargent and Weinman (34) repeated Kawahata’s (18) experiments on women at different times of the menstrual cycle, but they reported that sweating onset did not vary as a function of the menstrual-cycle phase. Thus experimental results indicate that neither ES nor...
menstrual-cycle phase influences SR. However, it is possible that our forearm sweating data do not represent whole body sweating, although, in a second set of experiments (unpublished observations) in which whole body sweating was measured, we again observed no differences between ES and placebo groups.

Skin temperature varies as a function of ambient temperature, and the effect of local skin temperature on the sudomotor (27) and vasomotor (43) responses to exercise are well known. In this study, the estradiol-treated group tended to have a lower Tsk throughout the experiment, but no significant difference was found between groups at any time (Fig. 1). Both groups showed a fall in Tsk at the onset of exercise followed by an increase as Tse rose. The reduction in Tsk is attributed to a transient active cutaneous vasoconstriction (19). Because Tse was not changed during rest by ES in the present study, the Tse threshold for initiation of cutaneous vasodilation and SR was not changed.

Effect of ES on HSP 70 synthesis. The HSP 70 family is essential for cellular survival of heat stress, and its induction by nonheat stress or by genetic manipulation is sufficient to protect cells from thermal injury (26). In the present study, 3 days of ES did not alter HSP 70 accumulation in blood mononuclear cells. This may be attributed to subject-to-subject variability in the degree of stress encountered under the exercise protocol, and thus in the changes in HSP seen, or to the tissue specificity of the HSP 70 response (26). Estradiol significantly elevated HSP 70 and HSP 90 protein concentrations in the ventromedial hypothalamus of gonadectomized adult female rats (28). It is possible that the leukocytes used in the present study were not tissue specific to induce HSP 70 synthesis by ES. Another factor to be considered is that HSP 72 is very sensitive to heat stress. Ryan et al. (33) showed that HSP 70 synthesis was only induced when Tse was elevated above 40°C in human subjects. Locke et al. (23) demonstrated that synthesis of HSP 72 was induced in lymphocytes, spleen cells, and soleus muscle after 20 min of exercise with Tse rising above 40°C. However, in the present study, Tse only reached 38.1°C, possibly an insufficient thermal stress to induce HSP 70 synthesis.

In conclusion, at the level of stress employed in this study (60% VO2max, ambient temperature = 25°C, 50% RH), 3 days of high circulating levels of estradiol (1,000 pg/ml) have no effect on core temperature or thermoregulatory responses in young women during the follicular phase of their menstrual cycles. These results are supported at the cellular level by a lack of change in HSP 70 concentration with ES in these subjects. In future studies, a greater thermoregulatory stress and a crossover design, in which each subject serves as her own control, would reduce variability and improve chances for identifying a significant effect of estrogen, if it exists. Although the estrogen dose employed in this study was developed and tested for safety in in vivo fertilization programs, a lower dose would be more physiological.

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