Chronic hormone replacement therapy alters thermoregulatory and vasomotor function in postmenopausal women

E. M. Brooks, A. L. Morgan, J. M. Pierzga, S. L. Wladkowsk, J. T. O’Gorman, J. A. Derr, and W. L. Kenney. Chronic hormone replacement therapy alters thermoregulatory and vasomotor function in postmenopausal women. J. Appl. Physiol. 83(2): 477–484, 1997.—This investigation examined effects of chronic (≥2 yr) hormone replacement therapy (HRT), both estrogen replacement therapy (ERT) and estrogen plus progesterone therapy (E+P), on core temperature and skin blood flow responses of postmenopausal women. Twenty-five postmenopausal women [9 not on HRT (NO), 8 on ERT, 8 on E+P] exercised on a cycle ergometer for 1 h at an ambient temperature of 36°C. Cutaneous vascular conductance (CVC) was monitored by laser-Doppler flowmetry, and forearm vascular conductance (FVC) was measured by using venous occlusion plethysmography. Iontophoresis of bretylium tosylate was performed before exercise to block local vasoconstrictor (VC) activity at one skin site on the forearm. Rectal temperature (T rect) was lower for the ERT group (P < 0.01) compared with E+P and NO groups at rest and throughout exercise. FVC: mean body temperature (Tb) and CVC: Tb curves were shifted leftward for the ERT group (P < 0.01). Baseline CVC was significantly higher in the ERT group (P < 0.05), but there was no interaction between bretylium treatment and groups once exercise was initiated. These results suggest that 1) chronic ERT likely acts centrally to decrease T rect, 2) ERT lowers the T rect at which heat-loss effector mechanisms are initiated, primarily by actions on active cutaneous vasodilation, and 3) addition of exogenous progestins in HRT effectively blocks these effects.

Skin blood flow; vasodilation; temperature regulation; core temperature; reproductive hormones; estrogen; progesterone

IN HUMANS, skin blood flow (SkBF) is controlled by a noradrenergic vasoconstrictor (VC) system and an active vasodilator (VD) system. At rest in a thermoneutral environment, SkBF is primarily under the influence of tonic VC tone. However, during heat stress, human SkBF increases to effectively transfer heat from core to skin by an initial withdrawal of VC and a progressive activation of VD. Vasomotor control of the cutaneous circulation is important in maintaining core temperature (Tc) homeostasis in humans at rest as well as during thermal challenges.

There is substantial evidence that female reproductive hormones may directly or indirectly influence SkBF and thermoregulation. In young women, differential patterns in SkBF and thermoregulatory responses to heat stress and exercise occur during a normal menstrual cycle (14, 34). Compared with the follicular phase, the luteal phase of the menstrual cycle is associated with an elevation in body Tc of ~0.5°C coupled with an increased Tc threshold for the onset of heat-loss-effector function, including skin vasodilation (4, 14, 34). These patterns of blood flow and thermoregulatory responses have been attributed to elevated circulating progesterone (P4) or the increased P4/estrogen (E2) ratio that characterizes the luteal phase of the menstrual cycle (16).

While the preceding observations have suggested a role for endogenous P4 in vasomotor and thermoregulatory control, acute (14–23 days) exogenous estrogen replacement therapy (ERT) in postmenopausal women causes a leftward shift in the curve relating cutaneous blood flow and Tc, and increased thermotolerance (36). Tc was decreased at baseline and throughout exercise after acute ERT. Results from animal studies are consistent with the preceding observations in women (1, 25). For example, when Baker and co-workers (1) administered E2 to ovariectomized (OVX) rats, a lowered Tc and a reduced Tc threshold for heat loss (e.g., evaporative water loss through saliva spreading) resulted. In a more recent study, E2 administration to OVX rats decreased basal Tc and increased thermotolerance within 8–12 days of ERT administration (25). Thus it appears that E2 enhances thermotolerance through a decreased Tc threshold for skin vasodilation and a lower regulated Tc, whereas P4 is associated with increased metabolic heat production and an elevated Tc (14). There is evidence of reproductive hormones acting at both central and peripheral levels (6, 26, 33). However, the precise mechanism(s) through which reproductive hormones alter thermoregulatory responses to heat stress and exercise is not clear.

Although previous studies have examined SkBF and thermoregulatory changes in response to heat stress and exercise in premenopausal women during the menstrual cycle (4, 14, 34) and in postmenopausal women on acute ERT (36), the effects of chronic (functionally defined here as continuous therapy of ≥2 yr) ERT and estrogen plus progesterone therapy (E+P) have not been investigated. Acute administration of ERT may result in an expansion of plasma volume (PV), which can potentially impact thermoregulatory function and SkBF patterns (10, 11, 36). Thus thermoregulatory alterations with acute ERT could be due to changes in PV rather than to direct effects of E2 on the central nervous system or on cutaneous vessels. We theorized that acute increases in PV previously observed with ERT (36) would return to baseline levels by 2 yr, thus allowing for a comparison of direct effects of hormone replacement therapy (HRT) on thermoregulatory function.
Therefore, the primary purpose of this study was to examine the influence of chronic ERT and E+P on thermoregulation and control of SkBF during exercise in the heat in three groups of women [no hormone therapy (NO), ERT, and E+P]. We hypothesized that chronic ERT would result in a lowered Tc and a reduced Tc threshold at which heat-loss-effector mechanisms would be regulated and that E+P would attenuate these changes. A second goal of this study was to determine the efferent mechanism through which chronic ERT and E+P act to alter cutaneous vasomotor control. By selectively blocking skin VC through local iontophoresis of bretylium tosylate at one site in the forearm, it was possible to examine and identify the peripheral sympathetic pathway(s) (VC vs. VT) through which E2 and P4 may act to alter the pattern of SkBF control during heat stress and exercise.

METHODS

Subjects. The present investigation was approved in advance by the Institutional Review Board of the Pennsylvania State University. After a detailed explanation of the procedures, nine postmenopausal women in the NO group, eight postmenopausal women receiving chronic oral ERT, and eight postmenopausal women receiving chronic oral E+P were recruited. Women were defined as postmenopausal by one or more of the following criteria: 1) complete cessation of menses for ≥1 year after a history of amenorrhea, 2) hysterectomy and oophorectomy, or 3) 2-wk repeat serum E2 concentration ([E2]) ≥30 pg/ml. Women who participated were no longer experiencing symptoms (hot flashes, insomnia, and so forth) normally associated with the perimenopausal period. Chronic HRT was functionally defined as continuous therapy for ≥2 years. All but five women on HRT received 0.625 mg of Premarin (Wyeth-Ayerst Laboratories, Philadelphia, PA) on a daily basis. The five exceptions included three women who received 0.625 mg of Premarin on the first 25 days of the month; a fourth woman who received 0.625 mg of Premarin on Monday, Wednesday, and Friday; and a fifth woman who received 0.625 mg of Premarin on all odd days and 1.25 mg of Premarin on even days. One of these women also used a vaginal estrogen cream. P4 dosages ranged from 2.5 to 10 mg, and like E2, the pill cycle varied among women. Progesterone agents included Provera (Upjohn, Kalamazoo, MI) and Cycrin (Esi Lederle, Philadelphia, PA), both of which contain medroxyprogesterone acetate. One woman in the E+P group received daily E2 but received 400 mg of P4 per day (medroxyprogesterone acetate, Paddock Laboratories, Philadelphia, PA) for 14 days every third month.

All subjects were screened by a physician, and body adiposity was estimated from skinfold thickness measurements at seven skin sites (pectoral, triceps, midaxillary, abdomen, thigh, suprailiac, subcapular). To determine peak aerobic power (V̇O2peak), subjects performed a graded exercise test on a modified cycle ergometer during which heart rate (HR) was recorded by an electrocardiogram, and blood pressure was measured by brachial auscultation. Body surface area (A S ) was calculated from height and weight (7), and physical activity level was estimated by using a validated questionnaire (6). Venous blood samples collected 5 min before exercise on the day of the experimental trial were assayed for estradiol-17β (E2) and P4. Subject characteristics are presented in Table 1, and serum hormone concentrations are presented in Table 2.

Table 1. Physical characteristics of subjects, activity level, resting plasma volume, and sweating rate

<table>
<thead>
<tr>
<th>Group</th>
<th>NO</th>
<th>ERT</th>
<th>E+P</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>59±2</td>
<td>54±2</td>
<td>58±2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>160±2</td>
<td>162±2</td>
<td>162±2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>61±2</td>
<td>72±6</td>
<td>68±4</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>30±2</td>
<td>34±3</td>
<td>35±3</td>
</tr>
<tr>
<td>Activity</td>
<td>31±4</td>
<td>21±4</td>
<td>19±6</td>
</tr>
<tr>
<td>V̇O2peak, mL·kg⁻¹·min⁻¹</td>
<td>24±4.5</td>
<td>20.0±1.7</td>
<td>22.6±1.9</td>
</tr>
<tr>
<td>PV, ml/kg</td>
<td>38.6±1.4</td>
<td>34.9±3.2</td>
<td>40.2±2.1</td>
</tr>
<tr>
<td>Sweating rate, g·m⁻²·h⁻¹</td>
<td>243±31</td>
<td>233±56</td>
<td>214±38</td>
</tr>
</tbody>
</table>

Values are means ± SE. NO, no hormone replacement therapy; ERT, estrogen replacement therapy; E+P, estrogen plus progesterone therapy; n, no. of subjects; A S , body surface area; V̇O2peak, peak O2 uptake; PV, plasma volume. Measurement of activity is based on the scale found in DiPietro et al. (8).

Criteria for exclusion of subjects included 1) an abnormal electrocardiogram during the graded exercise test, 2) hypertension (resting systolic pressure >140 mmHg or diastolic pressure >90 mmHg), 3) smoking, 4) any diagnosed metabolic or cardiovascular disease, or 5) taking of any medication with the potential to influence thermoregulatory or cardiovascular variables of interest.

Preexperimental procedures. The study was performed between the months of December and July, with no effort to artificially acclimate the subjects to the heat. Randomization of testing order minimized the potential for any systematic seasonal effect. Subjects reported to the lab between 0700 and 1000 on the morning of the exercise protocol. Pretest instructions included 1) no alcohol for 48 h, 2) no caffeine for 12 h, 3) no strenuous exercise for 12 h, and 4) consumption of an extra liter of water during the 24 h preceding the test.

Experimental procedures. On arrival of subjects at the laboratory, bretylium tosylate iontophoresis was locally performed to block VC at two sites on the right forearm (20), with the second site providing an alternate. In case the VC blockade at the first site was incomplete. Bretylium tosylate (100 mM) was dissolved in doubly distilled (18.3 MΩ·cm) water (NAPure, Barnstead, Dubuque, IA) and iontophoresed for 40 min over a 3-cm² area of skin by using alternating current (Lectro Patch, General Medical, Los Angeles, CA). Doubly distilled water was iontophoresed over a third site on the right forearm (3-cm² area) to serve as a control. After this procedure, each subject drank 5 ml water/kg body weight to ensure adequate hydration before exercise.

Approximately 1 h later, blockade of VC at bretylium-treated (BT) sites was verified by using whole body cooling as a stimulus for reflex skin VC. The subject inserted a rectal thermistor, provided a urine sample, dressed in exercise clothing (shorts and sports bra), and was fitted with a water-perfused suit (Diving Unlimited, San Diego, CA) covering the entire body except for the hands, feet, and head. After stable baseline measurements of HR, laser-Doppler flux

Table 2. Venous concentrations of estradiol and progesterone of subjects by group

<table>
<thead>
<tr>
<th>Group</th>
<th>Estradiol-17β, pg/ml</th>
<th>Progesterone, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>24.3±1.1</td>
<td>0.31±0.01</td>
</tr>
<tr>
<td>ERT</td>
<td>160.5±16.2*</td>
<td>0.34±0.06</td>
</tr>
<tr>
<td>E+P</td>
<td>99.2±30.4*</td>
<td>0.27±0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significantly different from NO (P<0.05).
humidity and LDF were monitored continuously throughout this baseline and exercise (described below). Arm blood flow (FBF) was recorded at 2-min intervals during baseline and exercise (described below).

After verification of VC blockade, the water-perfused suit was removed and the subject rested on the cycle ergometer while additional probes and monitors were attached. The environmental chamber was warmed to dry-bulb temperature = 36°C and wet-bulb temperature = 24°C (relative humidity = 40%). After ~5 min were allowed for stabilization, baseline measurements were collected for 10 min. Mean arterial pressure (MAP), HR, Tre, mean skin temperature (Ts_k), and LDF were monitored continuously throughout this 10-min baseline and the exercise period that followed. Forearm blood flow (FBF) was recorded at 2-min intervals during baseline and exercise (described below).

After the baseline period, subjects exercised for 30 min at 40% VO_{peak} then 30 min at 60% VO_{peak}. Each subject initially cycled at 60 revolutions/min at a resistance of 30 W, and resistance was increased by 30 W every 2 min until the target intensity was reached. After subjects completed 1 h of exercise, resistance was decreased, and subjects cycled slowly to maintain blood pressure. By using thermostatically heated probe holders, local Ts_k at the laser-Doppler probe sites was then increased to 42.5–43.0°C and maintained for ~40 min to obtain a site-specific maximal LDF. Maximal LDF was verified by performing a postocclusion reactive hyperemia maneuver (18).

Measurements. Tre was measured by using a series 400 Yellow Springs Instruments rectal thermistor inserted 10 cm past the anal sphincter. Ts_k was calculated as the weighted average of temperatures recorded by thermocouples (type T; Omega Engineering, Stamford, CT) affixed to four uncovered skin sites: chest, upper arm, thigh, and calf (29). Mean body temperature (T_b) was calculated as T_b = 0.8 Ts_k + 0.2 Ts_35 (35). MAP and HR were continuously monitored from a Finapres plethysmograph with the use of a mercury-in-Silastic strain gauge (EC4 Plethysmograph; Hokanson, Bellevue, WA) (38). During heating and dynamic leg exercise, increases in FBF are confined to the forearm skin rather than the underlying muscle (5, 19). An occlusion cuff (Hokanson) around the wrist was inflated to suprasystolic (200 mmHg) pressures to occlude hand blood flow, while an upper arm cuff cycled between 10 s of inflation (40–60 mmHg) and 5 s of deflation during measurement cycles (E20 Rapid Cuff Inflator, Hokanson). The FBF at each time point comprised the mean of a series of four readings initiated at 2-min intervals. Forearm vascular conductance (FVC = FBF/MAP) was reported in units of milliliters per 100 milliliters per minute per 100 millimeters mercury and later was plotted as FVC: Ts_k.

As described above, changes in SkBF were also examined using laser-Doppler flowmetry (model DRT4 laser blood flow monitor; Moor Instruments, Devon, UK). LDF was recorded at a BT and a control site from probes attached to the right forearm by using the aforementioned thermostatically controlled holders. Cutaneous vascular conductance (CVC) was calculated as LDF/MAP. Because LDF is highly variable between skin sites within the same individual as well as between different individuals (2), CVC at each skin site was standardized by expressing CVC as a percentage of the maximal CVC at that skin site (%CVC_{max}) obtained during local heating of the site to 42.5–43.0°C (37).

T_re individual T_sk, MAP, and HR data were each collected at 5 data points/s, averaged over 1-min intervals by using a SuperScope II (GW Instruments, Somerville, MA) data-acquisition system, and stored on a dedicated computer (Macintosh Quadra 650, Apple Computer, Cupertino, CA). Similarly, LDF data were recorded at a rate of 1 data point/s, and a mean was calculated for 1-min intervals.

A nude body weight was recorded for each subject before and after completion of each session. An estimate of sweating rate (in g·h^{-1}·m^{-2}) was calculated from the change in body weight, corrected for urine volume production but not for respiratory water loss (assumed to be negligible).

Venous blood samples were collected (SST Vacutainer; Becton-Dickinson, Rutherford, New Jersey) ~5 min before exercise during the experimental trial, stored in ice, and centrifuged. Serum was frozen and later assayed in duplicate. Estradiol-17β concentration was measured from serum aliquots with an I^{212} labeled double-antibody radioimmunoassay (RIA) procedure (ICN Biomedicals, Costa Mesa, CA). The sensitivity of the assay was 9 pg/ml, and inter- and intra-assay precision coefficients of variation were <12% and <11%, respectively, for an estradiol range of 28–38 pg/ml. Progesterone concentrations were measured at the Milton S. Hershey Medical Center Core Endocrine Laboratory by RIA with the use of an antibody-coated tube methodology. Assay sensitivity was 0.10 ng/ml, and inter- and intra-assay precision coefficients of variation were both <10% for a P_{4} range of 0.7–1.0 ng/ml.

Three to six days after the exercise protocol, subjects returned to the laboratory for measurement of resting PV by Evans blue dye dilution. Subjects arrived early at the laboratory after a 12-h overnight fast. Subjects rested in a seated position for at least 15 min at normothermia, then a 20-ml control blood sample was obtained through a heparinized butterfly needle. Approximately 3.0 g of dye were injected, then blood was collected at 10-, 20-, and 30-min postinjection. Plasma samples were later analyzed spectrophotometrically at a wavelength of 620 nm (Spectronic 21D, Milton Roy, Rochester, NY). Reported values (Table 1) are based on the peak absorbance reading, which occurred at 10 min for all subjects.

Analysis of data. Data are presented as means ± SE. Descriptive plots of T_sk, HR, change in T_re, T_re, MAP, and T_b vs. exercise time were analyzed as follows. The independent variable (exercise time) was partitioned into seven regularly spaced bins of 4-min width and with a gap of 6 min between bins. Within each subject, the dependent variables were averaged within the range of each bin for time, and a repeated-measures analysis of variance model was fit to the data. “Group” was the between-subjects factor, and “binned time” was the within-subjects factor.

The descriptive plots of %CVC_{max} vs. T_b showed a general sigmoid shape, as previously described (21). A set of four-parameter sigmoid curves was fitted to the data by using a nonlinear mixed-effects model that permits estimation of separate parameters for each individual and condition (22). The four-parameter model has the form

\[
\%CVC_{\text{max}} = (A_{1} - A_{2})[1 + (T_{\text{so}}/C_{1})^{-1}]^{-1} + D_{1}
\]

where, for the ith subject, A_{i} is the maximum, B_{i} is the slope parameter governing the steepness of the sigmoid curve, C_{i} is the effective temperature at 50% of CVC_{max} (ET_{50}), and D_{i} is the minimum. A separate repeated-measures analysis of variance model was then used to examine the effect of group
and condition on each of the four parameters of the sigmoid curve.

The descriptive plots of FVC vs. T\textsubscript{b} showed a functional relationship that had certain broadly consistent features among curves but could not be readily modeled by a sigmoid curve. Instead, four independent raters identified four features of each masked plot: a baseline, a threshold, a slope, and a plateau. The average of the estimates from each rater had interrater reliabilities (39) of 0.97, 0.94, 0.94, and 1.0 for the baseline, threshold, slope, and plateau, respectively. The average estimate of each feature was used as the dependent variable in the repeated-measures analysis of variance, as described above. A one-way analysis of variance was performed to examine among-group differences in subject characteristics (see Table 1) and venous hormone concentrations (Table 2).

For all analyses, an \( \alpha \) of 0.05 was used as the criterion for statistical significance of factors and their interactions. Follow-up tests with a Bonferroni correction were used to evaluate the significance of specific pairwise comparisons.

**RESULTS**

As illustrated in Fig. 1, T\textsubscript{re} was significantly lower in the ERT group compared with E + P and NO groups at rest and throughout exercise (\( P < 0.05 \)). Once exercise was initiated, the rate and magnitude of increase in T\textsubscript{re} during exercise were not significantly different among the three groups. A similar relationship existed for T\textsubscript{b} during exercise which was not significantly different among groups (Fig. 2). The T\textsubscript{b} threshold for the onset of cutaneous vasodilation in women taking ERT (36.5 ± 0.1°C) was significantly (\( P < 0.05 \)) lower than that for NO and E + P groups (\( P = 0.0001 \)) during exercise.

Table 3 presents the HR, MAP, and T\textsubscript{sk} responses at rest and the end of 30 min at each exercise intensity. HR and T\textsubscript{sk} were not different among the three groups at rest or during exercise.

The curve relating FVC to T\textsubscript{b} was shifted to the left for the ERT women compared with the remaining groups, but the slope and plateau of the FVC: T\textsubscript{b} curve was not significantly different among groups (Fig. 2). The T\textsubscript{b} threshold for the onset of cutaneous vasodilation in women taking ERT (36.5 ± 0.1°C) was significantly (\( P < 0.05 \)) lower than that for NO group (T\textsubscript{b} = 36.9 ± 0.1°C). Baseline FVC was not significantly different among the three groups of women (8.1 ± 0.8, 7.1 ± 0.4, and 8.3 ± 0.4 ml·100ml\textsuperscript{-1}·min\textsuperscript{-1}·100mmHg\textsuperscript{-1} for ERT, NO, and E + P groups, respectively; \( P < 0.05 \)). Therefore, during the early rise phase of FVC, FVC was higher in the ERT group than in NO and E + P groups because of a shift in the T\textsubscript{b} threshold.

**Table 3.** Selected group physiological responses at rest (time 0) and at end of 30 min at each exercise intensity

<table>
<thead>
<tr>
<th>Exercise Intensity</th>
<th>NO</th>
<th>ERT</th>
<th>E + P</th>
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<tbody>
<tr>
<td>HR, beats/min</td>
<td>84 ± 4</td>
<td>81 ± 4*</td>
<td>89 ± 4</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>86 ± 5</td>
<td>86 ± 5</td>
<td>89 ± 3</td>
</tr>
<tr>
<td>T\textsubscript{sk}, °C</td>
<td>97 ± 7</td>
<td>98 ± 5</td>
<td>87 ± 5</td>
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</table>

Values are means ± SE. T\textsubscript{sk}, skin temperature; T\textsubscript{b}, mean body temperature; HR, heart rate; MAP, mean arterial pressure; 40 and 60% \%VO\textsubscript{2}peak. *Significantly different from NO and E + P, \( P < 0.05 \).

Fig. 1. Mean rectal temperature (T\textsubscript{re}) at rest and during exercise at 40% peak O\textsubscript{2} consumption (VO\textsubscript{2}peak) and 60% VO\textsubscript{2}peak in an ambient temperature of 36°C. Baseline period consists of -10 to 0 min. Exercise began at 0 min and was completed at 60 min. T\textsubscript{re} of estrogen replacement therapy (ERT) group was significantly lower than estrogen plus progesterone therapy (E + P) and no hormone replacement therapy (NO) groups at baseline and throughout exercise (\( P < 0.05 \)); n, no. of subjects. Bars represent 1 SE.

Fig. 2. Forearm vascular conductance (FVC), calculated as forearm blood flow divided by mean arterial pressure, is plotted against mean body temperature (T\textsubscript{b}) calculated as T\textsubscript{b} = 0.8 T\textsubscript{re} + 0.2 skin temperature for each of 3 groups. Curves relating FVC to T\textsubscript{b} were shifted significantly (\( P < 0.05 \)) to left in ERT group compared with E + P and NO groups, with no change in slope. Bars represent 1 SE.
The present investigation provided insight into the influence of chronic HRT (ERT and E+P) on thermoregulatory function and cutaneous vasomotor control in postmenopausal women. The primary finding was that chronic ERT significantly reduces the regulated baseline $T_c$ by altering vasomotor function, but the addition of progestins to HRT blocks this thermoregulatory effect (Figs. 1–3). The similar resting PV values (Table 1) among the groups verify our premise that acute increases in PV with ERT (36) are no longer evident after 2 yr, and the PV values effectively rule out hypervolemia as a primary mechanism. This finding suggests that the thermoregulatory adjustments observed are more likely caused by direct effects of reproductive hormones on the thermoregulatory centers in the preoptic area anterior hypothalamus (PO/AH), the vasculature, or both. Although the modifications in thermoregulatory function and SkBF observed in the present study are more likely central in nature, the differential effect of BT on baseline $\%CVC_{max}$ in ERT suggests that peripheral actions by reproductive steroid hormones on the vasculature also may occur (Table 4). Finally, the similarity of the slope of the $\%CVC_{max}$ vs $T_b$ curve and the plateau in $\%CVC_{max}$ curve between BT and control skin sites in all three groups suggests that VD sensitivity is not altered by exogenous reproductive hormones.

$T_c$ regulation. Heat balance is achieved by equivalent rates of heat production and heat loss. At neutral (24–25°C) ambient temperatures at rest, human $T_c$ is regulated by alterations in SkBF rather than by changing metabolism appreciably or evaporative cooling (13, 32). $T_{re}$ is maintained at ~37°C, while $T_{sk}$ may vary from ~33 to 35°C (13) in this so-called “vasomotor” zone. SkBF in this zone is regulated by adjustments in VC tone, such that SkBF ranges from 2 to 6 ml·min⁻¹·100 ml·skin⁻¹. Although these changes in SkBF are rather small, they can have rather profound effects on heat transfer, and therefore, on resting $T_c$ (17).

As mentioned previously, control of thermoregulatory SkBF at rest and during exercise is altered in eumenorheic women during the menstrual cycle (4, 14, 34). A recurrent observation is a significant elevation in resting $T_c$ and in the $T_c$ threshold (by ~0.5°C) for the initiation of cutaneous vasodilation and sweating during the luteal phase of the menstrual cycle, a time when the $P_4$ to $E_2$ ratio is significantly increased. It is generally believed that $P_4$ is thermogenic (14) and alters the control of heat loss effectors to increase $T_c$ and that $E_2$ unopposed by $P_4$ decreases $T_c$ (16). For
example, $T_{re}$ in premenopausal women at rest and throughout exercise is lowest immediately before ovulation, intermediate during menses, and highest during the midluteal phase (4). Thus, the ratio of the concentrations of the reproductive hormones is an important determinant of the level at which $T_c$ is regulated.

Our laboratory previously investigated the effects of acute (2–3 wk) ERT on thermoregulatory and SkBF responses to exercise in the heat in postmenopausal women (36). We found a significant decrease in $T_{re}$ and esophageal temperature ($T_{es}$) at rest and during exercise after ERT. Furthermore, in that study, the $T_{es}$ threshold for the onset of vasodilation was reduced. Like acute ERT, chronic ERT significantly increased serum [E$_2$] in postmenopausal women compared with the NO group ($P < 0.05$, Table 2), significantly reduced $T_{re}$ and $T_b$ at rest and throughout exercise, and produced a leftward shift in the curves relating SkBF to $T_b$. The reduced level at which $T_c$ is regulated in the ERT group throughout exercise is likely caused by vasomotor adjustments rather than changes in sensible or insensible heat loss, because exercise sweating rate was not significantly different among groups (Table 1). The combination of these two studies suggests that the thermoregulatory advantages associated with ERT are achieved within 2–3 wk and are maintained throughout the duration of continuous ERT administration. However, it is unknown how quickly these benefits are lost if ERT is discontinued.

In the present study, serum [E$_2$] was significantly higher in E+P than in NO ($P < 0.05$, Table 2). Although the addition of progesterone to HRT attenuated the thermoregulatory effects of ERT, it did not cause $T_c$ to increase beyond that observed for NO. Because a minimal quantity of P$_4$ (2.5–10 mg) is incorporated into commercial HRT, the serum P$_4$ concentration (P$_4$) resulting from E+P therapy is low. In fact, there was no difference in serum [P$_4$] among the three groups of women. We attribute these results to the time of venous blood collection (6 h after pill ingestion), clearance rates of P$_4$, the low cross-reactivity between the assay and medroxyprogesterone acetate (oral progestin), and potential individual differences in the metabolism and adrenal production of steroid hormones. It is possible that increasing doses of P$_4$ could further increase $T_c$ and the level at which $T_c$ is regulated (31).

The lower regulated $T_c$ and the leftward shift in the $T_b$ threshold for cutaneous vasodilation in the ERT group, along with the inhibition of these thermoregulatory responses with the addition of progesterins in HRT, are consistent with an alteration of thermoregulatory function by reproductive hormones via a central mechanism. Estradiol stimulates warmth-sensitive neurons in PO/AH tissue slices of the rat (33). Additionally, Nakayama and Suzuki (26) examined the effects of intravenous P$_4$ administration on the activity of thermosensitive neurons in the hypothalamus of the rabbit and noted that P$_4$ increased the firing rate of cold-sensitive neurons while concurrently decreasing the firing rate of warm-sensitive neurons. However, in that study (26), because P$_4$ was not directly applied to thermosensitive neurons, it was unclear whether P$_4$ directly stimulated the neurons or if P$_4$ activated an additional cellular mediator that then acted on thermosensitive neurons in the hypothalamus.

Reproductive steroid hormones could act centrally by crossing the blood-brain barrier and directly stimulating thermosensitive neurons in the PO/AH. Androgen, E$_2$, and P$_4$ receptors have been characterized and mapped within the rat brain (24). However, it is also possible that these steroids act indirectly by stimulating a secondary mediator or pathway. E$_2$ and P$_4$ have been shown to differentially stimulate cytokine and prostaglandin secretion in a dose-dependent manner (3, 9). For example, Flynn (9) noted that lower doses of E$_2$ and P$_4$ ($\sim 10^{-9}$ M and $\sim 10^{-7}$ M, respectively) stimulated interleukin-1 (IL-1) production from monocytes, but higher doses ($\geq 10^{-8}$ M and $\geq 10^{-6}$ M, respectively) inhibited production. Similarly, Polan and coworkers (28) more recently noted biphasic dose-response curves for IL-1 activity by E$_2$ and P$_4$.

Cannon and Dinarello (3) noted that, during the luteal phase of the menstrual cycle in women, a profound increase occurred in the plasma activity of IL-1, a mediator of fever. The luteal phase of the menstrual cycle is similar to fever in that $T_c$ is regulated at a higher temperature. The measurement of the agonist-to-antagonist ratio is important for determining the effective response of cytokines. For example, the ratio of IL-1$\beta$ to IL-1 receptor antagonist (IL-1RA) was found to be elevated in women during the luteal phase of the menstrual cycle compared with women in the follicular phase (23). In postmenopausal women, cytokine production is inconsistent. Pacifici et al. (27) observed elevated cytokine bioactivity in the circulation, including IL-1$\beta$ and IL-6, after menopause, and a reduction in these cytokines after the initiation of E+P. However, not all investigators have reported similar findings (15). Inconsistent results could be due to the dosage of E$_2$ or P$_4$, time past the onset of menopause, health of the subject, and methodology. In summary, thermoregulatory alterations by reproductive steroids could be due to direct actions by these hormones at the PO/AH, indirect effects by other cellular mediators, such as cytokines, or both.

SkBF. At rest in thermoneutral environments, $T_c$ is regulated by vasomotor adjustments rather than metabolic changes. However, beyond thermoneutrality, vasomotor alterations along with other thermoregulatory effectors (e.g., shivering and sweating) are initiated. During exercise and heat stress, heat storage requires that SkBF be significantly increased by withdrawal of VC and activation of the VD system to convect heat from the core to the skin for dissipation. Reflex increases in SkBF are driven by increases in both $T_c$ and $T_{sk}$. In the present study, $T_b$ was calculated to account for the contributions of both thermal drives on heat-loss-effector function, including SkBF (35). Thus, the SkBF responses were plotted as a function of $T_b$ rather than $T_{re}$.
That control of SkBF is altered by hormonal status is clearly illustrated by the leftward shift in SkBF: $T_b$ curves in the ERT group (Figs. 2 and 3) compared with NO and E+P groups ($P < 0.05$, Table 4). Based on previous studies (1, 33), these observations are likely caused by a central alteration in the regulated level of $T_c$. Because the slopes of the %CVC max: $T_b$ curves at BT and control skin sites were not significantly different within or between groups (Table 4), nor was the rate of rise in FVC: $T_b$ curve different among groups, end-organ sensitivity to VD does not seem to be altered by reproductive hormones once exercise is initiated. Finally, CVC reached a similar percentage of the site-specific maximal conductance at both BT and control skin sites in all three groups. Because VC activity was blocked by BT, the plateau in CVC during exercise must be due to a limit in VD that appears not to be dependent on hormonal status.

An interesting and unexpected finding in the present investigation was the significant ERT group and treatment interaction for %CVC max at baseline (Table 4). One would expect CVC at rest to be greater at a site where basal VC activity had been blocked, especially because it is assumed that only the VC system is activated at thermoneutral resting conditions. However, baseline %CVC max at control sites in the ERT group was significantly higher than that in the other groups, and higher than the %CVC max at BT sites within the ERT group. This phenomenon could have a major effect on resting $T_c$ for reasons initially discussed in $T_c$ regulation and may help explain the lower resting $T_c$ in the ERT group. Because %CVC max is a function of absolute CVC and the site-specific CVC max, the combination of ERT and BT could potentially alter either of these parameters. Intra-arterial infusion of estradiol-17β has been shown to potentiate endothelium-dependent vasodilation (12) and to increase basal coronary flow in postmenopausal women (30). However, it is unknown whether HRT administration in postmenopausal women similarly alters maximal cutaneous flow. A decrease in CVC max seems unlikely, given the vasodilatory nature of E2. The second alternative to explain the higher resting %CVC max at control skin sites in the ERT group is consistent with the idea that E2 is sympathetic inhibitory at a central level, i.e., a central thermoregulatory action. In this case, the reduced %CVC max at BT skin sites in the ERT group may be due to an inhibitory effect by BT on a vasodilatory factor or pathway that is stimulated by E2 at rest. The convergence of %CVC max: $T_b$ curves at the BT and control skin sites in the ERT group suggests that this hypothetical vasodilatory factor or pathway plays an insignificant role once VD is activated. Potential vasodilatory mechanisms by estradiol include increased production or activity of nitric oxide, increased prostaglandin production, stimulation of potassium channels, inhibition of calcium channels, or blockade of vasoconstrictor agents such as endothelin (8). The mechanism(s) through which BT interacts with ERT is unknown at present.

Another effect of BT iontophoresis was a small but consistent rightward shift in $ET_{50}$ within each group. There was no group-by-treatment interaction, because $ET_{50}$ at BT sites was consistently shifted to the right in each of the three groups. Possible explanations for this finding are 1) the onset of active vasodilation may be delayed by BT or 2) VC withdrawal may play a role at the onset of vasodilation, but this effect can only be seen during slow heating of an individual. This finding raises questions about additional cellular actions of bretylium tosylate.

In conclusion, chronic ERT in postmenopausal women reduced the regulated $T_c$ at rest and during exercise. The addition of progestins to HRT attenuated the thermoregulatory effects of E2, such that the E+P group responded to exercise in the heat as did the NO group. There may be an interactive effect of BT and ERT, such that the average resting %CVC max is significantly higher at control skin sites in the ERT group than at BT sites in the ERT group or at either site in the other two groups. However, during exercise, this differential effect is overcome by an equivalent sensitivity to increasing VD activity in the three groups along with achievement of a similar %CVC max.

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