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

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Brooks, E. M., A. L. Morgan, J. M. Pierzga, S. L. Wladkowski, 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_{re}) was ~0.5°C lower for the ERT group ($P < 0.01$) compared with E+P and NO groups at rest and throughout exercise. FVC: mean body temperature (T_b) and CVC: T_b curves were shifted ~0.5°C leftward for the ERT group ($P < 0.0001$). 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_{re}, *2*) ERT lowers the T_{re} 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 (T_c) 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 T_c of ~0.5°C coupled with an increased T_c 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 (P_4) or the increased P_4/estrogen (E_2) ratio that characterizes the luteal phase of the menstrual cycle (16).

While the preceding observations have suggested a role for endogenous P_4 in vasomotor and thermoregulatory control, acute (14–23 days) exogenous estrogen replacement therapy (ERT) in postmenopausal women caused a leftward shift in the curve relating cutaneous blood flow and T_c during exercise in the heat (36). T_c 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 E_2 to ovariectomized (OVX) rats, a lowered T_c and a reduced T_c threshold for heat loss (e.g., evaporative water loss through saliva spreading) resulted. In a more recent study, E_2 administration to OVX rats decreased basal T_c and increased thermotolerance within 8–12 days of ERT administration (25). Thus it appears that E_2 enhances thermotolerance through a decreased T_c threshold for skin vasodilation and a lower regulated T_c, whereas P_4 is associated with increased metabolic heat production and an elevated T_c (14). There is evidence of reproductive hormones acting at both central and peripheral levels (8, 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 E_2 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 T_c and a reduced T_c 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. VD) through which E_2 and P_4 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 eumenorrhea, *2*) hysterectomy and oophrectomy, or *3*) 2-wk repeat serum E_2 concentration ($[E_2]$) ≤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. P_4 dosages ranged from 2.5 to 10 mg, and like E_2, 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 E_2 but received 400 mg of P_4 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, subscapular). To determine peak aerobic power ($\dot{V}O_{2peak}$), 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_d) 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 and P_4. 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*

	NO	ERT	E+P
n	9	8	8
Age, yr	59 ± 2	54 ± 2	58 ± 2
Height, cm	160 ± 2	162 ± 3	162 ± 2
Weight, kg	61 ± 2	72 ± 6	68 ± 4
Body fat, %	30 ± 2	34 ± 3	35 ± 3
A_d, m^2	1.67 ± 0.07	1.67 ± 0.03	1.71 ± 0.06
Activity	31 ± 4	21 ± 4	19 ± 6
$\dot{V}O_{2peak}$, $ml \cdot kg^{-1} \cdot min^{-1}$	24.4 ± 0.5	20.0 ± 1.7	22.6 ± 1.9
PV, ml/kg	38.6 ± 1.4	34.9 ± 3.2	40.2 ± 2.1
Sweating rate, $g \cdot m^{-2} \cdot h^{-1}$	243 ± 31	233 ± 56	214 ± 38

Values are means ± SE. NO, no hormone replacement therapy; ERT, estrogen replacement therapy; E + P, estrogen plus progesterone therapy; *n*, no. of subjects; A_d, body surface area; $\dot{V}O_{2peak}$, peak O_2 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 (NANOpure, Barnstead, Dubuque, IA) and iontophoresed for 40 min over a 3-cm^2 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-cm^2 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*

Group	Estradiol-17β, pg/ml	Progesterone, ng/ml
NO	24.3 ± 1.1	0.31 ± 0.01
ERT	160.5 ± 16.2*	0.34 ± 0.06
E + P	99.2 ± 30.4*	0.27 ± 0.03

Values are means ± SE. *Significantly different from NO ($P < 0.05$).

(LDF) at control and BT sites, and rectal temperature (T_{re}) were established, whole-body cooling was performed for 3 min. Blockade of VC was considered complete if LDF either increased or remained stable at the BT site but decreased at the control site. If blockade was incomplete, the alternative BT site was tested in a similar manner. The experiment proceeded only if full blockade of VC by bretylium iontophoresis was achieved.

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, T_{re}, mean skin temperature (T_{sk}), 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% $\dot{V}O_{2peak}$, then 30 min at 60% $\dot{V}O_{2peak}$. 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 T_{sk} 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. T_{re} was measured by using a series 400 Yellow Springs Instruments rectal thermistor inserted 10 cm past the anal sphincter. T_{sk} 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 $T_b = 0.8\ T_{re} + 0.2\ T_{sk}$ (35). MAP and HR were continuously monitored from a Finapres cuff (Finapres blood pressure monitor, model 2300; Ohmeda, Louisville, CO) attached to the middle finger of the right hand.

FBF was measured on the left forearm by venous occlusion plethysmography 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: T_b.

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 a rate of 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 $g \cdot h^{-1} \cdot m^{-2}$) 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^{125}-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

$$\%CVC_{max\,i} = (A_i - D_i) \cdot [1 + (T_{b\,i}/C_i)B_i]^{-1} + D_i$$

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_b showed a functional response 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 α 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_{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_{re} during exercise were not significantly different among the three groups. A similar relationship existed for T_b (Table 3), i.e., the T_b of the ERT group was significantly lower than that for NO and E+P groups ($P = 0.0001$) during exercise.

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

The curve relating FVC to T_b was shifted to the left for the ERT women compared with the remaining groups, but the slope and plateau of the FVC: T_b curve was not significantly different among groups (Fig. 2). The T_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 the NO group (T_b = 36.9 ± 0.1°C). Baseline FVC was not significantly different among the three groups of women (8.1 ± 1.0, 7.1 ± 0.8, and 8.3 ± 9 ml·100 ml^{-1}·min^{-1}·100 mmHg^{-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_b threshold.

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

Exercise Intensity	NO	ERT	E+P
	T_{sk}, °C		
Rest	34.4 ± 0.2	34.3 ± 0.2	34.3 ± 0.2
40%	35.1 ± 0.2	34.7 ± 0.1	35.3 ± 0.2
60%	35.6 ± 0.3	35.1 ± 0.4	35.9 ± 0.4
	T_b, °C		
Rest	36.7 ± 0.1	36.4 ± 0.1*	36.6 ± 0.1
40%	37.0 ± 0.1	36.6 ± 0.1*	37.1 ± 0.1
60%	38.0 ± 0.2	37.5 ± 0.2*	38.1 ± 0.2
	HR, beats/min		
Rest	68 ± 4	68 ± 3	77 ± 3
40%	114 ± 6	105 ± 5	123 ± 4
60%	149 ± 8	138 ± 6	151 ± 5
	MAP, mmHg		
Rest	84 ± 4	81 ± 4*	89 ± 4
40%	86 ± 4	86 ± 5	89 ± 3
60%	97 ± 5	98 ± 5	87 ± 5

Values are means ± SE. T_{sk}, skin temperature; T_b, mean body temperature; HR, heart rate; MAP, mean arterial pressure; 40 and 60%, %$\dot{V}O_{2peak}$. *Significantly different from NO and E+P, $P < 0.05$.

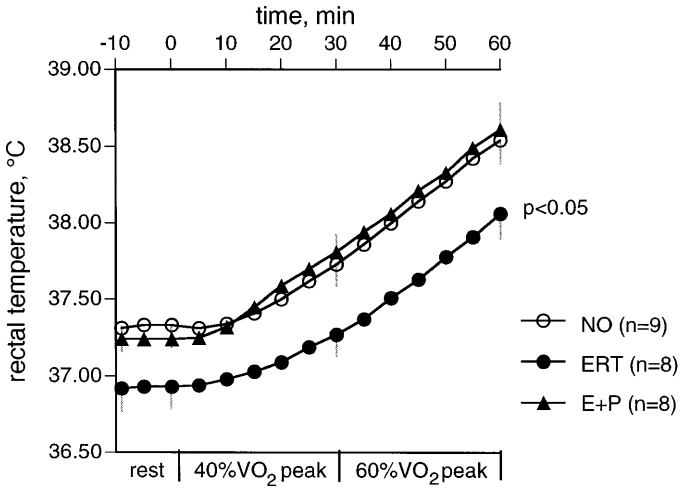

Fig. 1. Mean rectal temperature (T_{re}) at rest and during exercise at 40% peak O_2 consumption ($\dot{V}O_{2peak}$) and 60% $\dot{V}O_{2peak}$ in an ambient temperature of 36°C. Baseline period consists of −10 to 0 min. Exercise began at 0 min and was completed at 60 min. T_{re} of estrogen replacement 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.

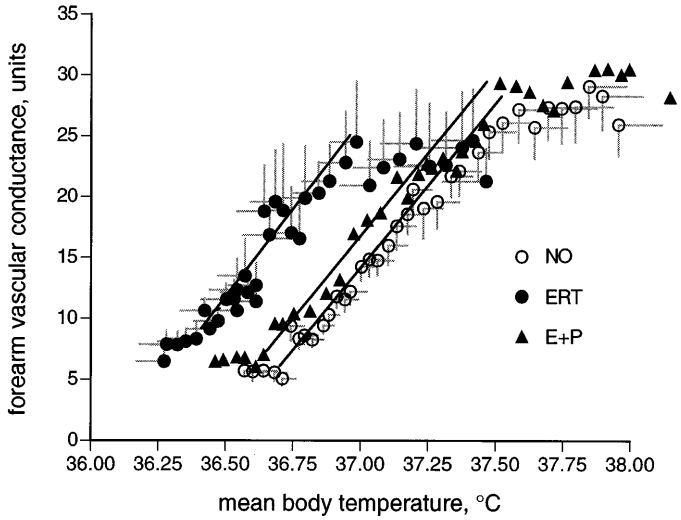

Fig. 2. Forearm vascular conductance (FVC), calculated as forearm blood flow divided by mean arterial pressure, is plotted against mean body temperature (T_b), calculated as T_b = 0.8 T_{re} + 0.2 skin temperature for each of 3 groups. Curves relating FVC to T_b were shifted significantly ($P < 0.05$) to left in ERT group compared with E+P and NO groups, with no change in slope. Bars represent 1 SE.

Table 4. *Parameter estimates for 4-parameter sigmoid model of %CVC$_{max}$ vs. T$_b$ for each group at control skin sites and sites pretreated with bretylium tosylate*

	A (max)	B (slope factor)	C (ET_{50})	D (min)
		Control sites		
NO	68 ± 4	−510 ± 102	36.9 ± 0.1[a]	9 ± 3[c]
ERT	68 ± 3	−623 ± 142	36.6 ± 0.10[a,b]	21 ± 3[c]
E + P	67 ± 3	−387 ± 61	37.1 ± 0.1[b]	12 ± 2
		Bretylium-treated sites		
NO	65 ± 4	−561 ± 75	37.1 ± 0.1* [d]	13 ± 3
ERT	63 ± 3	−526 ± 111	36.7 ± 0.1* [d,e]	9 ± 3*
E + P	64 ± 5	−394 ± 50	37.2 ± 0.1* [e]	13 ± 4

Values are means ± SE. Model takes form $\%CVC_{max\,i} = (A_i - D_i) \cdot [1 + (T_{b\,i}/C_i)B_i]^{-1} + D_i$. %CVC $_{max}$, % maximal cutaneous vascular conductance; *i*, *i*th subject; A is maximum parameter; B is slope factor; C is ET_{50} (effective temperature at 50% CVC_{max}); and D is minimum parameter. See *Analysis of data*. *Within-group differences for bretylium-treated vs. control groups are significantly different at $P < 0.05$. Means with like superscripts are significantly different at $P < 0.05$ for among-group comparisons.

Table 4 presents the parameter estimates for the sigmoid model of $\%CVC_{max}$: T_b at both control and BT skin sites for each group, and this relationship is plotted in Fig. 3 for control skin sites. Baseline $\%CVC_{max}$ was not significantly different among groups at BT sites. However, at control sites, baseline $\%CVC_{max}$ in ERT was significantly higher ($P < 0.05$) than that of the NO group. As in FVC: T_b, there was a leftward shift in the $\%CVC_{max}$: T_b curve in the ERT group. This observation is supported by a significantly lower ET_{50} at both BT and control skin sites for the ERT group compared with that of the E+P and NO groups ($P < 0.05$). The slope of $\%CVC_{max}$: T_b did not differ among the three groups, nor was it affected by BT treatment. In all groups, CVC reached a similar percentage of the site-specific maximum (59–68%) at high T_c regardless of BT treatment. As presented in Table 4, BT significantly affected the ET_{50} by shifting it slightly to the right within each group. Therefore, although BT affected baseline $\%CVC_{max}$ in ERT, there was no interaction between group and treatment once exercise began.

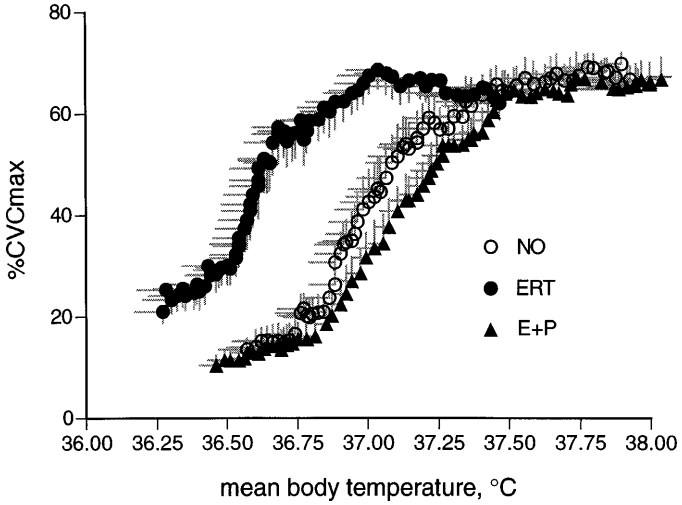

Fig. 3. Cutaneous vascular conductance (CVC), calculated as laser-Doppler flux divided by mean arterial pressure and expressed as %maximal CVC ($\%CVC_{max}$), at control skin sites is plotted against T_b (see Fig. 2). Like FVC, $\%CVC_{max}$-T_b curve was shifted significantly to left in ERT group compared with E+P and NO groups ($P < 0.05$). Resting $\%CVC_{max}$ in ERT was significantly higher than in E+P and NO groups ($P < 0.05$). However, once exercise was initiated, there was no significant difference in slope of $\%CVC_{max}$-T_b curve or $\%CVC_{max}$ plateau between 3 groups. Bars represent 1 SE.

DISCUSSION

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 $\%CVC_{max}$: 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 blood·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 eumenorrheic 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 progestins 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 warmth-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 (~10^{-9} M and ~10^{-7} M, respectively) stimulated interleukin-1 (IL-1) production from monocytes, but higher doses (≥10^{-8} M and ≥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 healthy premenopausal 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 about 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-1β to IL-1 receptor antagonist (IL-1Ra) was found to be elevated in women during the luteal phase of the menstrual cycle compared with men or 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-1β 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 E_2. The second alternative to explain the higher resting $\%CVC_{max}$ at control skin sites in the ERT group is consistent with the idea that E_2 is sympathoinhibitory 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 E_2 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 E_2, 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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