Effects of hormone replacement therapy on hemodynamic responses of postmenopausal women to passive heating

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Received 16 August 1999; accepted in final form 29 February 2000

Dunbar, Stacey L., and W. Larry Kenney. Effects of hormone replacement therapy on hemodynamic responses of postmenopausal women to passive heating. J Appl Physiol 89: 97–103, 2000.—To examine the influence of chronic hormone replacement therapy (HRT) on the central and peripheral cardiovascular responses of postmenopausal women to direct passive heating, seven women taking estrogen replacement therapy, seven women taking estrogen and progesterone therapy, and seven women not taking HRT were passively heated with water-perfused suits to their individual limit of thermal tolerance. Measurements included heart rate (HR), cardiac output, blood pressure, skin blood flow, splanchnic blood flow, renal blood flow, esophageal temperature, and mean skin temperature. Cardiac output was higher in women taking estrogen and progesterone therapy than in women not taking HRT (7.12 ± 0.70 vs. 5.02 ± 0.57 l/min at the limit of thermal tolerance, respectively; P < 0.05) because of a higher HR. However, when the HR data were plotted as a percentage of the maximum HR or percentage of HR reserve, there were no differences among the three groups of women. Neither splanchnic nor renal blood flow differed among the groups of women. These data suggest that HRT has little effect on the cardiovascular responses to direct passive heating.

aging; cardiac output; skin blood flow; cardiovascular responses; hyperthermia

DURING HEAT STRESS, blood flow to the cutaneous vasculature increases dramatically to move heat away from the core and dissipate it to the environment. To support this increase in skin blood flow (SkBF), cardiac output (Qc) increases, and blood is distributed away from the splanchnic and renal vascular beds.

Our laboratory recently reported that, during passive heat stress to the limit of thermal tolerance, older men (64–81 yr), compared with younger men (19–28 yr), responded with a lower SkBF, which was associated with both a lower Qc and a reduced ability to redistribute blood from their combined splanchnic and renal vascular beds (12). The lower Qc in the older men was due primarily to a lower stroke volume (SV).

Numerous studies have shown that both endogenous and exogenous female steroid hormones can affect cardiovascular and thermoregulatory function in young women. Both during a normal menstrual cycle and during oral contraceptive use, changes in core temperature and the SkBF response to heating correspond to changes in the estrogen-to-progesterone ratio (2, 18).

There is also evidence that hormone replacement therapy (HRT) in postmenopausal women may affect the cardiovascular mechanisms that underlie human thermoregulatory control. Women taking estrogen replacement therapy (ERT) have lower core temperatures at rest and during exercise in a warm environment compared with women not taking any HRT (1, 19). Additionally, women taking ERT have a higher SkBF at any given core temperature than women not taking any HRT (1). These alterations due to ERT were blocked by the addition of progestins to the HRT.

It is unknown, however, how control of central hemodynamic function and blood flow to visceral organs may be affected by HRT during direct passive heating. In several animal models, ERT has been shown to cause increased Qc via increased SV (7, 9, 16). Additionally, in humans, Qc is increased during pregnancy, a time when estrogen levels are elevated (10, 13). In a recent study of postmenopausal women, Qc, SV, and ejection fraction (EF) were increased at rest after 16 wk of estrogen and progesterone (E+P) replacement therapy (17). Although an increase in plasma volume (PV) could have accounted for the increase in SV in that study, it could not account for the increase in EF. Thus there is evidence that HRT can increase resting Qc by increasing the inotropic function of the heart. An increase in inotropic function of the heart could attenuate the age-related decline in the Qc response to direct passive heating.

HRT also has the potential to impact peripheral hemodynamic responses to heating. HRT in postmenopausal women has been shown to increase blood flow to several vascular beds, including the heart and uterus (6). It is unknown whether HRT also increases blood flow to the splanchnic and renal vascular beds and whether it would affect the vasoconstriction that occurs in these vascular beds during a heat stress. For example, more vasoconstriction in the viscera may be

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needed to support the increase in cutaneous blood flow seen with ERT.

Therefore, the purpose of this investigation was to examine the influence of chronic ERT and E+P on the central and peripheral hemodynamic responses to prolonged direct passive heating. It was hypothesized that women taking ERT and E+P would have a higher Q, at the limit of thermal tolerance because of a higher SV.

METHODS

Subjects

All procedures used in this investigation were approved in advance by the Committee for the Protection of Human Subjects of the Office of Regulatory Compliance at The Pennsylvania State University. After approved informed consent procedures, 21 postmenopausal women (aged 52–80 yr) volunteered for the present investigation. Seven women were not receiving HRT (No HRT), seven women were receiving chronic ERT, and seven women were receiving chronic E+P. Chronic ERT was defined as continuous therapy for at least 2 yr. All but one woman taking ERT received 0.625 mg of Premarin (Wyeth-Ayerst Laboratories, Philadelphia, PA). The one exception was a woman using an estrogen patch (Estraderm, Novartis Pharmaceuticals, East Hanover, NJ). Four of the women in the E+P group were receiving PremPro (Wyeth-Ayerst Laboratories), which contains 0.625 mg of Premarin and 2.5 mg medroxyprogesterone acetate (Provera, Upjohn, Kalamazoo, MI). The other three women in the E+P group received 0.625 mg of Premarin for the first 25 days of the month and received either 5 mg (2 women) or 10 mg (1 woman) of Provera on days 14–25. Women who were not receiving HRT were defined as postmenopausal by the following criteria: 1) circulating follicle-stimulating hormone >30 IU/ml (11), 2) circulating 17β-estradiol <25 pg/ml (11), and 3) cessation of menses for at least 1 yr. Women who had both a hysterectomy and oophorectomy were considered postmenopausal. All women were healthy nonsmokers not taking any medication that could affect the thermoregulatory or cardiovascular variables of interest.

Before participating in the experimental protocol, subjects underwent a screening procedure. This procedure included a medical examination by a physician, measurement of skinfold thickness as an estimate of adiposity, a resting 12-lead electrocardiogram, blood tests to establish normal liver and kidney function, and blood pressure measurement. Subjects also underwent a maximal graded exercise test on a treadmill to determine maximal heart rate (HR) and maximal oxygen consumption (VO₂ max).

Subject characteristics are presented in Table 1. The three groups of subjects were matched for height, weight, adiposity, surface area, VO₂ max, PV, and blood volume. The No HRT group was older than the E+P group and thus had a lower maximal HR. As expected, women taking ERT and E+P had higher serum estradiol concentrations and lower FSH concentrations than did the women in the No HRT group. Additionally, the initial esophageal temperature (Tes) measured during the first minute of the baseline period was lower in the ERT group than in either the E+P or No HRT groups.

Experimental Procedures

Instrumentation. Subjects reported to the laboratory at 0800 after an 8-h fast. They were asked to refrain from ingesting alcohol for 24 h before the test and caffeine for 12 h before the test and were encouraged to drink plenty of fluids in the 24 h before the experiment. Subjects were weighed before and after the experiment to estimate sweat loss. A catheter was inserted into an antecubital or forearm vein of the right arm for infusion of a solution containing indocyanine green (ICG) and p-aminohippurate (PAH) for the measurement of splanchic blood flow (SBF) and renal blood flow (RBF), respectively. A second catheter was placed in a vein distal to the site of infusion in either the left or right arm for blood sampling.

With assistance, the subjects inserted a thermistor located in a sealed pediatric feeding tube for measurement of Tes. To aid insertion, subjects drank 2.5 ml/kg of water. Eight copper-constantan thermocouples were affixed to the subjects’ skin on the upper and lower back and on the left side and right sides of the chest, stomach, shoulder, thigh, and calf. Mean skin temperature (Tsk) was calculated as the unweighted average of these eight thermocouples. Two blood pressure cuffs and a mercury-in-Silastic strain gauge were placed on the left forearm for measurement of forearm blood flow (FBF) via venous occlusion plethysmography (24). Under conditions of passive heating, increases in FBF represent increases in SkBF because underlying muscle blood flow does not change (3). The FBF measurement at each time point was calculated as the average of three to four traces. The upper blood pressure cuff was also used to measure blood pressure by brachial auscultation. HR was measured via a three-lead electrocardiogram.

P, was determined with the acetylene-rebreathing technique (20) by using a mass spectrometer for analysis of gas concentrations. SV was calculated as Q / HR. Total peripheral resistance (TPR) was calculated as mean arterial pressure (MAP) / Q.

Protocol. After instrumentation, subjects donned a water-perfused suit and plastic coverall to prevent evaporative cooling. Subjects wore only shorts and a sports bra under the suit. The water-perfused suit covered the entire body except for the head, feet, and arms below the elbow. Subjects were supine throughout the experiment, and, to begin the protocol, thermoneutral water (34°C) was circulated through the
suit for 45 min, during which baseline data were collected. After the baseline period, the temperature of the water circulating through the suit was changed to ~50°C, and this temperature was maintained until each subject's individual limit of thermal tolerance was reached. The limit of thermal tolerance was defined as either the point at which subjects expressed that they were too uncomfortable to continue or when $T_{es}$ reached 39°C. At this time, the subjects were cooled with 15°C water for at least 15 min.

$T_{es}$, $T_{sk}$, and HR were measured continuously throughout the protocol. All other measurements were taken every 15 min throughout the protocol. The measurement of $Q_s$ and a 7-ml blood draw were performed simultaneously, followed sequentially by FBF measurement and then blood pressure measurement.

**SBF and RBF.** In this study, SBF and RBF were measured from constant infusion of ICG and PAH, respectively. At the beginning of the baseline period, 20 ml of blood were drawn to serve as a blank. Then, a priming dose of ICG (0.10 mg/kg body wt) and PAH (8.0 mg/kg body wt) was injected into the infusion catheter. For the remainder of the protocol, a constant infusion of 0.5 mg/ml ICG and 12 mg/ml PAH was maintained at a rate of 1.0 ml/min. As described in Protocol, blood was drawn every 15 min after the start of the infusion for the entire protocol. Plasma concentration of ICG was measured spectrophotometrically (absorbency 805 nm), and plasma concentration of PAH was determined with the measured spectrophotometrically (absorbency 805 nm), and for the entire protocol. Plasma concentration of ICG was determined spectrophotometrically (absorbency of 805 nm). ER was calculated for each subject from the two slopes of the plasma disappearance curve of ICG. Heating does not appear to alter ER (14), and thus the ER determined on this day was used in the calculations of SBF during the heating protocol.

**Determination of PV**

On a day separate from the experimental protocol and the ER determination, baseline PV was determined via injection of Evans blue dye (EBD) (5). After the subjects had been supine for 30 min, an aliquot of venous blood was drawn as a spectrophotometer blank. Then, a bolus of EBD was injected into an antecubital vein. Venous blood samples were collected every 10 min for 30 min. Samples were centrifuged at 3,000 rpm for 20 min, and the plasma concentration of EBD was determined spectrophotometrically (absorbancy of 620 nm).

**Statistical Analyses**

Significant differences between subject characteristics were determined by using a one-way ANOVA. A two-way repeated-measures ANOVA (main effect of HRT group, main effect of time, and group $\times$ time interaction) was performed on all variables from baseline through the first 60 min of heating. To account for differences in the time to the limit of thermal tolerance, each individual's final 15 min of heating were also used to calculate a group mean, and another two-way repeated-measures ANOVA was performed on these data. Homogeneity of variance and normality of the data were verified before the ANOVA. Post hoc analyses with a Bonferroni correction were used to evaluate significance of specific pairwise comparisons. The level of significance was set at $P < 0.05$. Values are presented as means ± SE.

**RESULTS**

**Temperature Variables**

The time to reach thermal tolerance varied widely among individuals (45–105 min), but there was no significant difference among the three groups (69 ± 8, 71 ± 6, and 76 ± 7 min for E+P, ERT, and No HRT groups, respectively; $P = 0.77$). There were no significant group differences in $T_{es}$, or $T_{sk}$ at baseline or at any time during the heating protocol (Fig. 1). ERT has been shown to lower baseline $T_{es}$ (1, 19). Table 1 shows that, during the first minute of the baseline period, $T_{es}$ lower in the ERT group compared with both the No HRT and E+P groups. However, the water-perfused suit is designed to tightly control skin temperature, and the plastic coverall prevents evaporative heat loss. This experimental manipulation could have caused temperatures in the three groups of women to not differ statistically by the end of the baseline period. It is also possible that the sample size in this study was too small to detect the small difference in temperature. Sweating rate did not differ significantly among the three groups of women (442 ± 48, 444 ± 81, and 441 ± 32 g/h in ERT, E+P, and No HRT groups, respectively; $P = 0.99$).
Cardiac Responses

Q˙c (Fig. 2) was significantly increased in all three groups of women after 30 min of heating (P < 0.05). The E+P group had higher Q˙c than did the No HRT group throughout the entire protocol (7.12 ± 0.70 vs. 5.02 ± 0.57 l/min at the limit of thermal tolerance, respectively; P < 0.05). The higher Q˙c in the E+P group was due to a higher HR response (Fig. 2). HR in the E+P group was significantly higher than in both the ERT and No HRT groups beginning at 15 min of heating (107 ± 5 vs. 90 ± 3 and 86 ± 5 beats/min at the limit of thermal tolerance in E+P vs. ERT and No HRT groups, respectively; P < 0.05). HR was significantly elevated above baseline in all three groups of women beginning at 15 min of heating (P < 0.05). SV was not significantly different among the three groups at any time during the protocol, and SV did not change significantly during the heating (Fig. 2).

The higher HR response in the E+P group can be explained by their higher maximal HRs (Table 1). When the HR data are plotted either as a percentage of the maximal HR or as a percentage of the HR reserve (Fig. 3), there are no longer any statistically significant group differences in the HR response (63 ± 1, 57 ± 3, and 57 ± 3% of maximal HR at the limit of thermal tolerance in E+P, ERT, and No HRT groups, respectively; P = 0.29 for group effect, P = 0.19 for group × time interaction; 39 ± 4, 28 ± 4, and 30 ± 6% of HR reserve at the limit of thermal tolerance in E+P, ERT, and No HRT groups, respectively; P = 0.63 for group effect, P = 0.29 for group × time interaction).
Other Cardiovascular Variables

FBF (Fig. 4) increased significantly in all three groups of women after the first 15 min of heating \((P < 0.05)\), but there were no significant differences among the groups in FBF at any time during the protocol.

SBF (Fig. 4) was significantly higher in the E+P group than in the ERT or No HRT groups at baseline \((1,131 \pm 64 vs. 889 \pm 90 and 980 \pm 117 \text{ ml/min}, \text{respectively}; \ P < 0.05)\) but not during heating. SBF decreased significantly below baseline values for all groups after the first 15 min of heating \((P < 0.05)\). RBF (Fig. 4) decreased significantly after the first 15 min of heating in all three groups of women \((P < 0.05)\), but there were no significant differences in RBF among the groups at any time during the protocol.

MAP (Fig. 5) was well maintained during the heating in all three groups of women. There were no significant differences in MAP among the three groups at baseline \((100 \pm 4, 92 \pm 4, \text{and } 93 \pm 2 \text{ mmHg in E+P, ERT, and No HRT groups, respectively})\) or during heating \((100 \pm 4, 91 \pm 2, \text{and } 93 \pm 2 \text{ mmHg at the limit of thermal tolerance in E+P, ERT, and No HRT groups, respectively}; \ P = 0.12 \text{ for group main effect, } P = 0.37 \text{ for group } \times \text{ time interaction})\). TPR (Fig. 5) fell significantly during the protocol in all three groups of women \((P < 0.05)\). There were no significant differences in TPR among the three groups of women during the protocol \((14.7 \pm 1.2, 15.1 \pm 1.0, \text{and } 21.1 \pm 2.1 \text{ mmHg} \cdot \text{min}^{-1} \cdot \text{m}^{-2} \text{ at the limit of thermal tolerance in E+P, ERT, and No HRT groups, respectively}; \ P = 0.17 \text{ for group main effect, } P = 0.83 \text{ for group } \times \text{ time interaction})\).

DISCUSSION

The main finding of the present study was that chronic HRT, either ERT alone or in combination with...
Thus the higher $Q_c$ response in the E group was caused by a higher HR. However, the women in the E group were younger than the women in the No HRT group. Consequently, this higher HR was at a lower percentage of their HR reserve during passive heating, than did the women in the No HRT group. We believe that this increased HR response may put older individuals, especially those with coronary artery disease, at a greater risk for cardiac events during heat stress. Thus an increase in inotropic function of the heart could be beneficial during heat stress. We hypothesized that chronic HRT would increase $Q_c$ during direct passive heating by increasing SV (i.e., by increasing inotropic function).

In a previous study from our laboratory (12), the increase in $Q_c$ directed to the skin during direct passive heating was smaller in older men compared with younger men. SV fell in the older men, but not in the younger men, meaning that the older men had to rely more heavily on an increase in chronotropic function, and operated at a higher percentage of their HR reserve during passive heating, than did the younger men. We believe that this increased HR response may put older individuals, especially those with coronary artery disease, at a greater risk for cardiac events during heat stress.

Fig. 5. Mean arterial pressure (MAP) and total peripheral resistance (TPR) in 3 groups of postmenopausal women during last 15 min of baseline, first 60 min of direct passive heating, and averaged values during last 15 min of each subject's heating period. There were no differences among the 3 groups for either variable. Values are means ± SE. Dotted vertical line, start of heating. †Values under brackets are significantly different from baseline for all groups of women, $P < 0.05$.

progestins, does not affect the cardiac or hemodynamic responses of postmenopausal women to direct passive heating. The E+P group did have a higher $Q_c$ than did the No HRT group throughout the entire protocol because of a higher HR. However, the women in the E+P group were younger than the women in the No HRT group and thus had a higher maximal HR. Consequently, when the HR data were plotted as a percentage of the maximal HR, there were no significant differences observed among the three groups of women. Thus the higher $Q_c$ response in the E+P group seems to be an artifact of the group characteristics (specifically, age (12)) rather than an effect of the HRT. There were no differences in any of the other hemodynamic variables among the groups during the heating protocol, and, therefore, it seems that HRT does not affect the hemodynamic responses to direct passive heating.

It appears that the “extra” $Q_c$ in the E+P group was evenly distributed throughout the vascular beds examined in this study. The E+P group had the highest flows to the cutaneous, splanchnic, and renal vascular beds consistently throughout the entire protocol, although these group differences were not statistically significant (Fig. 3).

It has been consistently shown that ERT in postmenopausal women decreases core temperature at rest (1), during exercise in the heat (1, 19), and during passive heating (E. M. Brooks-Asplund, personal communication). These same studies showed that ERT could increase cardiac inotropic function (6, 9, 17, 22). However, in most of those studies, the estrogen administration was not prolonged enough to allow PV and blood volume to return to baseline. That is, for the first several months of ERT, PV is expanded. If ERT is continued beyond several months, PV returns to pre-ERT values. Thus the observed increases in PV and blood volume in the previous studies could have, at least partially, accounted for the observed increases in $Q_c$ and SV.

The present study also showed no differences between women taking HRT and women not taking HRT with respect to SBF and RBF. From these data, it does not appear that HRT increases resting blood flow to these vascular beds, in contrast to some other vascular beds, namely the heart and uterus (6). It also does not appear that HRT affects the vasoconstriction that occurs in these vascular beds during heating to redistribute flow to the skin.
caused a slight increase in skin and core temperatures in the ERT group and slight decreases in these temperatures in the other two groups of women. It is also possible that the sample size in this study was not large enough to detect the small difference in temperature that existed at the end of the baseline period.

Charkoudian and Johnson (2) have used a whole body heating protocol with a water-perfused suit and coverall. In contrast to our results, they did show thermoregulatory effects of estrogen in the form of oral contraceptives. However, they do not report the temperature of the water circulating through the suit, if any, during the baseline period. They also did not elevate skin or core temperatures as much as in this study. Additionally, they measured SkBF via laser-Doppler flowmetry as opposed to venous occlusion plethysmography used in this study. These protocol differences may explain the differences in results between their study and ours.

In summary, the purpose of this investigation was to examine the effect of HRT on the cardiac and hemodynamic responses during direct passive heating to the limits of thermal tolerance in postmenopausal women. There were no differences among the groups in blood flow to the skin or visceral circulation. Q˙c was higher in women taking E + P due to a higher HR, but this can be explained by a higher maximal HR in this group of women, a function of their slightly younger mean age.

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