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Differential Effects of Estrogen and Medroxyprogesterone on Basal and Stress-Induced Growth Hormone Release, IGF-1 Levels, and Cellular Immunity in Postmenopausal Women

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We evaluated the influence of continual estrogen replacement therapy (ERT) as presently practiced by postmenopausal women with conjugated estrogens and medroxyprogesterone acetate (MPA) on the growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis and cellular immunity. Thirty-nine postmenopausal women were evaluated (12 on no replacement, 14 on estrogen only, and 13 on estrogen and MPA). In the women receiving only conjugated estrogens, increased GH levels and decreased IGF-1 levels were found, which replicated previous research and probably reflected estrogen inhibition of hepatic IGF-1 production with a secondary increase in GH release because of reduced feedback inhibition. In women taking both MPA and estrogen, GH was increased and the previously observed estrogen induced decrease in IGF-1 levels was inhibited.

In order to determine the influence of ERT on psychosocial stress-induced GH release, math (mental stress) and speech (social stress) challenges were utilized, and they produced significant increases in heart rate in all three groups. The heart rate following stress was significantly enhanced by estrogen replacement. These stressors also led to increased GH secretion in the women taking estrogen and MPA, but not in the other two groups.

Gonadal steroids and GH can influence cellular immunity. We observed that ERT in both groups was associated with significantly enhanced lymphocyte responsiveness to the T-cell mitogens phytohemaglutinin (PHA) and Conconavalin A (Con A), and basal GH levels were correlated with the PHA response in the estrogen only group. ERT did not influence natural killer (NK) cell activity. We also found significant differences in the steady-state expression of latent Epstein-Barr virus (EBV) with increased antibody titers in the women in the estrogen only group and lower antibody titers in the MPA plus estrogen group. GH levels were correlated with EBV antibody titers in the estrogen plus MPA group.

This study supports the hypothesis that GH and immune modulation can be influenced by ERT in postmenopausal woman. Given the extant literature on the immune-enhancing effects of GH, these data suggest that ERT may slow the decline of GH secretion with aging, an event that has been implicated in immunosenescence.

Key Words: Estrogen; medroxyprogesterone; growth hormone; stress; IGF-1; immune; postmenopausal.

Introduction

Estrogen has been shown to stimulate growth hormone (GH) release, and its reduction in the postmenopausal period may accelerate the aging-induced decline in GH secretion in women *(1–3)*. When oral estradiol was administered to postmenopausal women, it stimulated GH release, but decreased insulin-like growth factor-1 (IGF-1) levels *(4–7)*. To explain this apparent paradox, it was suggested that estradiol was decreasing hepatic synthesis of IGF-1 and that serum GH increased because of a decrease in negative pituitary feedback *(4)*. We were interested in whether conjugated estrogens used commonly in estrogen replacement therapy (ERT) in postmenopausal women would induce similar changes in GH and IGF-1. We also wanted to determine if progesterone in the form of medroxyprogesterone acetate (MPA) would alter basal and/or stress-related GH secretion, since progesterone has been reported both to stimulate and inhibit tumoral GH release or have no effect on normal subjects' GH secretion *(8–10)*.

Also, we wished to investigate whether psychosocial stress as produced by mental and social stressors influ-

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Table 1
Participant Characteristics and Stress Reactivity for Control, Estrogen Only, and Estrogen plus Progestogen Groups[a]

	Control		Estrogen only		Estrogen + MPA	
Measure	*n*	Mean ± SEM	*n*	Mean ± SEM	*n*	Mean ± SEM
Age	12	62.75 ± 1.61[a]	14	59.71 ± 2.11[a]	13	60.77 ± 1.33[a]
BMI[b]	12	25.45 ± 0.91[a]	14	25.95 ± 1.02[a]	13	24.15 ± 1.05[a]
SHBG[c]	12	121.06 ± 21.36[a]	14	192.10 ± 27.53[b]	13	168.05 ± 20.69[a,b]
Years postmenopause[d]	12	14.25 ± 2.49[a,b]	14	17.78 ± 2.38[a]	13	10.15 ± 2.12[b]
Years ERT[e]	—	—	11	16.61 ± 3.26[a]	12	6.25 ± 1.43[b]
Premarin dosage[f]	—	—	13	0.41 ± 0.03[a]	11	0.37 ± 0.04[a]
Prestress HR[g]	12	62.44 ± 2.16[a]	13	64.19 ± 2.29[a,b]	13	68.60 ± 1.84[b]
Stress change HR	12	8.28 ± 1.72[a]	13	12.27 ± 2.11[a,b]	13	13.79 ± 1.67[b]

[a]SEM = standard error of mean. Means with different subscripts reading across the groups are significantly different at $p < 0.05$ or less.
[b]BMI = weight (kg)/(height [m]HR)^2.
[c]SHBG = sex hormone binding globulin, expressed in nmol/L.
[d]Number of years since last menses, whether natural or surgical.
[e]Number of years of continuous estrogen replacement up until present.
[f]Expressed as (Premarin [mg]/d/wt [lb]) × 10^2.
[g]HR = heart rate, expressed in beats/min.

enced GH levels and whether stress-enhanced GH secretion was affected by ERT. Utilizing examination stress *(11)*, marital conflict *(12)*, and math and speech stress (unpublished observations), we have seen variable GH responses. We have attributed this lack of GH response in part to inadequate sensitivity of the GH assays, an interpretation that can now be tested with the introduction of the newer methods used in this investigation.

Finally, sex steroids and GH influence cellular immunity *(13,14)*. Therefore, in the context of ERT, we examined the relation between GH/IGF-1 levels and several immune parameters, including natural killer (NK) cell cytotoxicity, and the blastogenic response of peripheral blood leukocytes (PBLs) to phytohemaglutinin (PHA) and Conconavalin A (Con A). We also examined the steady-state expression of latent Epstein-Barr virus (EBV) in women in these groups. It is well known that the cellular immune response plays a very important role in controlling the steady-state expression of latent herpesviruses, like EBV, and reactivation of the virus will induce increased levels of virus-specific antibody *(15)*.

Our study population consisted of 39 postmenopausal women, 12 were on no replacement therapy, 14 were on conjugated estrogens only, and 13 were on both conjugated estrogens and MPA. We used a well-established laboratory stress paradigm that employs math and speech stress tasks to evaluate the influence of estrogen and progesterone on GH reactivity to stress. An ultrasensitive chemiluminescence assay was used to measure GH *(16)*.

Results

Subject Characteristics and Stress Response

The control, estrogen only, and estrogen plus MPA groups were of similar age and BMI (Table 1). Premarin doses were similar in the replacement groups. The durations of ERT and years postmenopause were longer in the estrogen only group, because they had a surgically induced menopause. The impact of both of these variables was tested using analysis of covariance. Neither variable was significant, so they were dropped from further analyses.

SHBG levels were significantly increased over the control levels in the estrogen only group ($p < 0.05$), but not in the estrogen plus MPA group, suggesting that MPA was inhibiting estrogen stimulation of hepatic production of SHBG.

The math and speech stressors were effective in inducing physiological stress as evidenced by the increases in subject heart rate (Table 1). The greater heart rate reactivity seen in the estrogen groups has also been seen by others *(17)*.

The Influence of ERT on GH and IGF-1

Basal GH levels were higher in the women taking ERT, an effect that was enhanced by the addition of MPA ($p < 0.01$) (Fig. 1). The math and speech stress tasks did not enhance GH release in the control and estrogen only groups. In contrast, poststress levels of GH were three times higher in the MPA group than in the controls ($P < 0.05$) (Fig. 2), an effect that was observed 45 min after initiation of the stressors. Serum IGF-1 levels were significantly depressed in the estrogen group. However, there was no decrease in the MPA and estrogen individuals (Fig. 3).

The Influence of ERT on Immune Parameters

Estrogen significantly enhanced baseline mitogen stimulation of PBLs by both PHA ($p < 0.01$) and Con A ($p < 0.03$), which were higher in both the estrogen and MPA groups (Fig. 4). In the estrogen only group, the PHA-stimulated PBL values were significantly correlated (Spearman's $\rho = 0.54$, $p < 0.05$) with basal GH levels.

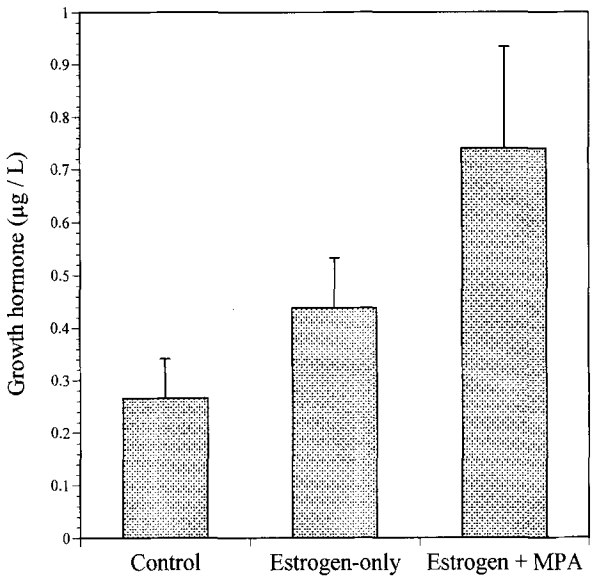

Fig. 1. Mean ± SEM baseline GH levels as a function of group. Estrogen therapy with or without MPA was associated with significantly ($p < 0.01$) increased baseline serum GH levels.

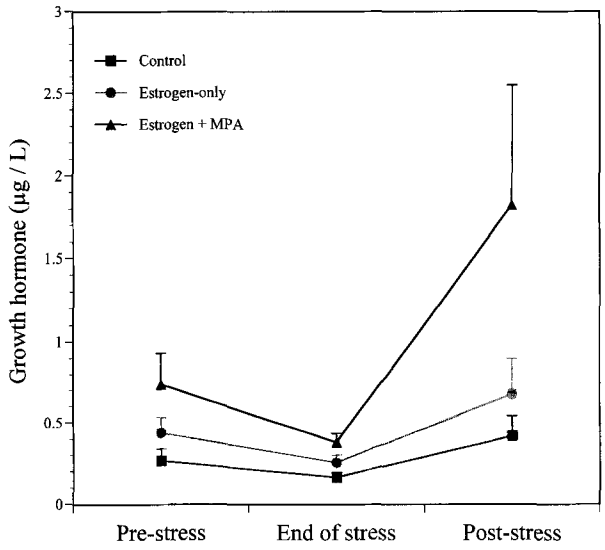

Fig. 2. The influence of stress on mean ± SEM GH as a function of group. Speech (social stress) and math (mental stress) challenges lasting approx 12 min produced a significant ($p < 0.05$) GH increase in only the estrogen plus MPA group.

Women in the estrogen only group had nonsignificantly higher EBV VCA IgG antibody titers than controls ($p < 0.08$), whereas the estrogen plus MPA group had significantly lower EBV antibody titers than the estrogen only group ($p < 0.05$), suggesting a suppressive influence of MPA on estrogen-associated changes in the steady-state expression of latent EBV (Fig. 5). Basal GH levels were significantly ($p < 0.05$) correlated with EBV antibody titers in the estrogen plus MPA group, suggesting that GH might be playing a role in the MPA inhibition of reactivation of latent EBV.

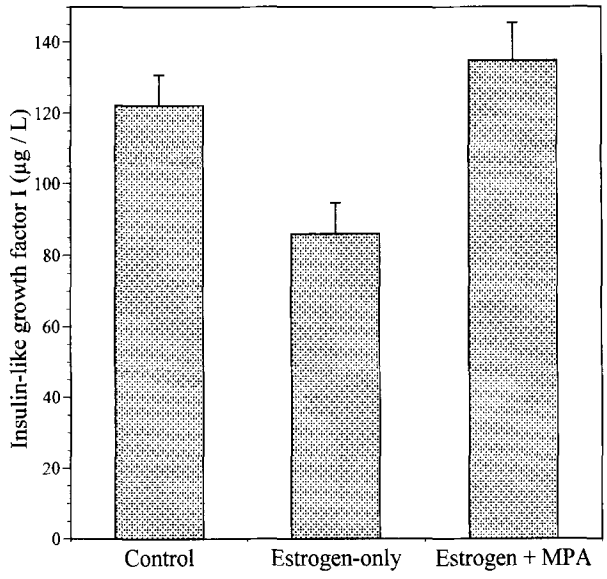

Fig. 3. Mean ± SEM IGF-1 levels as a function of group. Estrogen therapy was associated with significantly ($p < 0.01$) depressed baseline IGF-1 levels from control subjects, an effect that was inhibited by the addition of MPA.

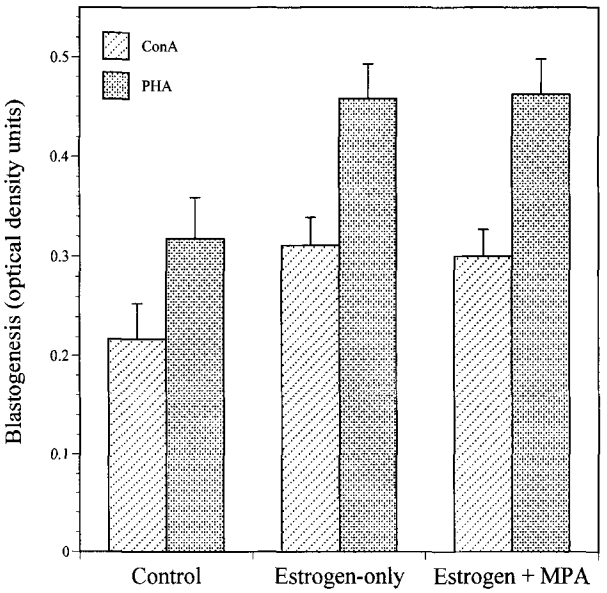

Fig. 4. The influence of mitogens on lymphocyte proliferation as a function of group. Estrogen therapy was associated with significantly ($p < 0.03$) enhanced baseline blastogenic response to Con A and PHA. This effect was not influenced by MPA.

NK cell cytotoxicity was not influenced by estrogen or basal GH levels, and group differences were not seen in the stress-induced NK cell cytotoxicity (data not shown).

Discussion

Previous investigations of the influence of estrogen on various physiological parameters, including endocrines, have used estradiol in short-term experiments *(18,19)*. In

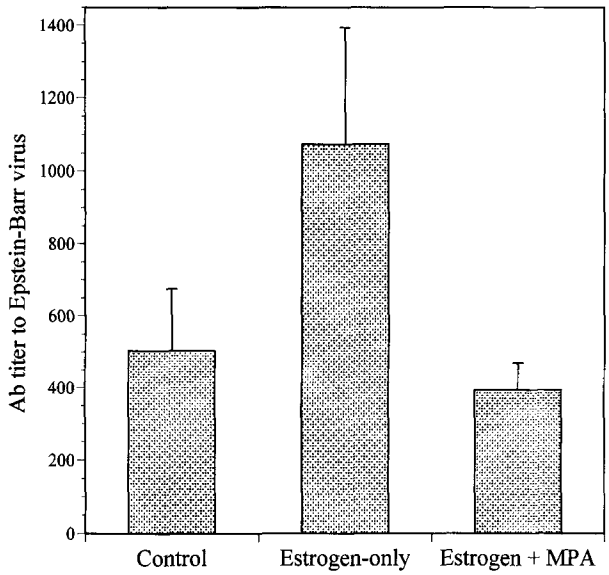

Fig. 5. Mean ± SEM baseline EBV titers as a function of group. MPA was significantly ($p < 0.05$) associated with lower baseline EBV VCA IgG antibody titers than the women in the estrogen only group.

this approach, serum levels of estradiol achieved with patch or oral replacement could be measured easily, whereas it is more difficult to ascertain the serum levels of the estrogenic components of conjugated estrogens. The objective of our study, however, was to evaluate the chronic influence of conjugated estrogens alone or associated with MPA on the GH/IGF-1 system, since they are commonly used in clinical practice. We utilized SHBG levels as a biological marker of estrogenic activity *(20)* and noted an appropriate increase in the estrogen alone group with nonsignificant blunting of this increase by MPA. This latter effect of MPA suggested a possible inhibitory influence of MPA on hepatic production of SHBG.

ERT had a significant relationship with the endocrine parameters examined in this study. Enhanced serum GH concentrations and decreased IGF-1 levels were associated with conjugated estrogens similar to what has been previously reported for oral or transdermal estradiol therapy *(4–7)*. These paradoxical findings have been explained by suggesting that estrogen inhibits hepatic production of IGF-1, and therefore, GH secretion increases because of diminished negative pituitary feedback *(3)*. This interpretation is supported by the observation that estrogen inhibits rodent hepatic IGF-1 mRNA generation *(21)*. In addition, there are a limited number of estrogen receptors in somatotropes *(22)*, and there is evidence that estrogen does not directly stimulate hypothalamic GHRH or inhibit hypothalamic somatostatin levels, the principal regulators of GH secretion *(23)*. In contrast, the women taking both MPA and estrogen had both elevated GH and IGF-1 levels. Therefore, MPA may have inhibited an estrogen-induced decrease in hepatic production of IGF-1.

We also evaluated the role of psychological stress on GH release, whether estrogen and MPA modified its secretion, and if these interactions can modulate different components of the cellular immune response. In previous investigations, we have used math (mental stress) and speech (social stress) to evaluate endocrine and immune reactivity *(24,25)*. In this study, these same stressors produced increases in heart rate similar to what we have shown in previous work *(24,25)*. The greater increase in heart rate in the ERT groups has also been seen by some investigators *(17)*, but not by others *(18,19)*. The mechanism responsible for this increase in heart rate presently is not clear.

In our earlier investigations, we have often failed to see significant stress-induced increases in GH release, which may have been related to the insensitivity of the GH assays employed *(11,12)*. In this study, however, using a sensitive chemiluminescent assay, we observed that math and speech stress did not significantly increase GH in the control women or the women using estrogen alone. In women on both estrogen and MPA, there was an increase in GH release, suggesting an enhancing effect of MPA on GH secretion.

In endocrinologic investigations, it is desirable to sample frequently over the interval of interest. This investigation, however, included the evaluation of several other hormones, additional immune parameters, and the stress protocol, which limited the number of GH samples that could be acquired over the 45 min of study. Although this limitation may have prevented us from demonstrating a stress-induced GH increase in the control and estrogen only groups, it did not prevent us from observing a significant increase in the estrogen plus MPA group. MPA has been shown to inhibit or stimulate GH release from pituitary tumors *(8,9)* and have no effect on GH secretion in normal individuals *(10)*. In this investigation, we did not test MPA independently; however, in concert with estrogen, it enhanced stress-induced GH release while preventing the estrogen-induced decrease in IGF-1 levels.

Gonadal steroids have been shown to have numerous effects on humoral and cellular immunity *(13,14)*. Estrogen and GH can influence immune system development in the thymus and bone marrow as well as on peripheral T- and B-cell function. Therefore, we evaluated some possible immune consequences of chronic ERT and examined whether these changes were correlated with GH/IGF-1 levels. PHA-induced lymphocyte blastogenesis was significantly enhanced in the ERT group. It was also significantly correlated with basal GH levels, but not serum IGF-1 concentrations in the estrogen-only group. Perhaps this correlation was produced by ERT-induced serum GH increases, which then stimulated lymphocyte IGF-1 levels *(26)*. MPA had no significant influence on these estrogen-mediated immune events, although progesterone has been shown to be immunosuppressive in certain settings *(27)*. These findings suggested that GH may have been involved in mediating this estrogen effect on lymphocyte blastoge-

nesis and are consistent with previous studies demonstrating an influence of GH on lymphocyte function *(14,26)*.

In people latently infected with herpesviruses like EBV, antibody titers to the virus reflect the steady-state expression of the latent virus. In this study, the women in the estrogen plus MPA group had the lowest antibody titers to latent EBV. The women in the estrogen only group showed a nonsignificantly higher level of EBV VCA IgG. Basal GH levels were correlated with EBV VCA IgG antibody titers in the women in the group using estrogen and MPA. Whether this is a result of the impact of GH on a component(s) important for controlling the replication of latent EBV directly or indirectly by modulating a component(s) of the cellular immune response important for controlling the replication of latent EBV remains to be determined. The health implications of the modulation of the steady-state expression EBV is not totally understood. Although little pathology appears to be associated with the reactivation of latent EBV in healthy individuals, there may be significant health risks for individuals who are immune-suppressed *(28)*.

It has been suggested that MPA may reduce some of the beneficial effects of ERT; for example, it may produce a more atherogenic lipid profile. In contrast, in this investigation, MPA appeared to potentiate estrogen's effect on GH by enhancing a stress-induced release while preventing the estrogen-related decrease in IGF-1 levels.

This study adds GH and immune effects to a growing literature regarding the multiple influences of ERT on the postmenopausal woman. Given the extant literature on the immune-enhancing effects of GH *(14,26)*, these data also suggest that ERT may slow the decline of GH secretion associated with aging, an event that has been implicated in immunosenescence *(29)*.

Methods

Participants

Thirty-nine women between 50 and 70 yr of age were recruited from the community by advertisement and were paid $75.00 for their participation (Table 1). In order to participate, the women had to meet the following criteria:

1. No menstrual periods for at least 2 yr.
2. Body mass index (BMI; calculated as weight in kg divided by squared height in m) <34.
3. A health history free from any disease with a clear cardiovascular, neuroendocrine, or immune component.
4. No diagnosed hypertension.
5. No use of cardiovascular prescription or nonprescription medication known to influence the endocrine or immune systems.
6. No tobacco usage.
7. On average, <10 caffeine and 10 alcohol drinks consumed/wk.
8. On average, <5 h of exercise/wk.
9. Adequate nutrition as determined by serum ferritin and albumin.
10. No speech, math, or needle phobia.
11. No current illness.

The women taking no estrogen replacement ($n = 12$) had not used any hormone replacement therapy for at least 10 yr; the group taking only an estrogen supplement ($n = 14$) had used Premarin on a daily basis for an average of 16 yr, and 13 women used both estrogen and MPA on a daily basis for an average of 6.25 yr. Our sample characteristics reflected current medical practice: 73% of the no ERT group and 100% of the estrogen plus progesterone group had no reproductive surgery, and 94% of the estrogen only group had hysterectomies.

Procedure

The day before participants came in for the study, they were reminded to refrain from exercising or consuming alcohol or nonprescription drugs during the day, and from eating or drinking anything other than water after midnight until their scheduled appointment. Participants were evaluated at either 7:45 or 10:15 in the morning (counterbalanced). When they arrived, the tasks and measures were reviewed, any questions were answered, and informed consent was obtained. The nurse then inserted a 20-gage indwelling catheter into an antecubital vein on the participant's arm. The participant then spent 20–25 min completing a set of questionnaires, and rested in a supine position for an additional 15 min to allow adaptation to the setting.

A baseline blood sample was acquired, and the participants received instructions for both of the stressor tasks. Any questions about these tasks were answered. The math and speech stressors were then administered in counterbalanced order, with the second stressor immediately following completion of the first. Electrocardiography was collected throughout the 3-min speech preparation, the 3-min speech delivery, and the 6-min serial subtraction task. Blood samples were collected immediately after the second stressor had been completed and after an additional 30 min.

The speech task was similar to that used by Saab et al. *(30)*. Participants responded to a hypothetical scenario in which they were treated unfairly. They were asked to cover a set of specific points, and to give intelligent and well-thought-out answers because their speeches would be recorded and compared with the speeches of other participants. They were given 3 min to prepare and 3 min to deliver their speeches, and were instructed to be sure to speak for the entire 3-min period *(24)*.

The participants also performed a 6-min mental arithmetic task similar to that used by Cacioppo et al. *(25)*. To maintain moderate task difficulty (i.e., approx 10 correct answers/min) and maximal task involvement, the subtrahend (number being subtracted) specified each minute was contingent on the participant's performance during the

preceding minute (e.g., better performance led to more difficult problems). Two measures for task performance were calculated: The number of subtractions attempted and percentage correct. These performance measures did not differ across groups.

Assays

The hormone assays were all performed in our laboratory using the blood samples drawn at the baseline and two poststress time periods. Plasma or serum samples were aliquoted and stored at –70°C until hormone assay. GH was measured by a sensitive chemiluminescence technique (Nichols Institute, San Juan Capistrano, CA) with intra- and interassay coefficients of variation (CVs) <8%. IGF-1 was determined by RIA (Nichols Institute) and sex hormone binding globulin (SHBG) (Diagnostic Systems Laboratories, Webster, TX) by IRMA. Both assays had intra- and interassay CVs of <10%.

Complete blood counts (CBCs) and differentials were carried out on each blood sample by the Clinical Immunology Laboratory at the Ohio State University Hospital using 5 mL of EDTA-treated blood. Mononuclear cells were obtained from 30 mL of heparinized blood on Histoplaque-1077 (Sigma Chemical Co., St. Louis, MO) density gradients, washed twice with magnesium- and calcium-free phosphate-buffered saline, counted in a Coulter counter (Coulter Electronics, Hialeah, FL), and then used immediately as described. The percentages of NK cells (CD56+) were determined using monoclonal antibodies (MAbs) (Coulter) by FACS analysis using routine procedures.

NK cell cytotoxicity was measured by incubation of PBLs using effector-to-target (E:T) cell ratios of 75:1, 37.5:1, and 18.75:1 with ^{51}Cr-labeled K-562 target cells, as previously described *(31)*. Activity was determined by the release of ^{51}Cr into the supernatant, which was measured using a Beckman 9000 γ-counter. NK cell cytotoxicity values were standardized at the 37.5:1 effector-to-target cell ratio with a logistic regression *(32)*.

Mitogen-stimulated PBL activity was assessed using the Cell Titer 96 AQ_{ueous} Non-Radioactive Cell proliferation Assay (Promega), which determines the number of viable proliferating cells by colorimetry. The assay is based on the conversion of the tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS), into a formazan that is soluble in tissue-culture medium. Ninety-six well plates were used to set up samples in triplicate, with Con A (Sigma) and PHA (Sigma) at final concentrations of 10.0, 5.0, and 2.5 μg/mL. A 50-μL aliquot of a cell suspension containing 1×10^6 cell/mL in RPMI-1640 medium supplemented with 5% fetal bovine serum (FBS) was added to wells of 96-well plates containing 50 μL of each mitogen dilution, or media (unstimulated controls): wells with media alone were used as background controls. The plates were incubated in an atmosphere of 5% CO_2 at 37°C, with humidity, for approx 68 h. After incubation, 20 μL of a 20:1 solution of MTS/phenazine methosulfate were added to the plates. The plates were then incubated for an additional 4 h, after which the optical density (OD) was recorded using a Titertek Multiscan MCC plate reader. Plasma antibody titers to EBV capsid antigen (VCA) IgG were determined by the indirect immunofluorescence test as previously described *(33)*.

Several questions guided our data analysis. We tested the effect of conjugated estrogens and MPA on the GH axis by comparing basal GH and IGF-1 levels among the three groups using analysis of variance (ANOVA). Effects of ERT on immune parameters were evaluated similarly; in addition, the correlations between immune variables and GH and IGF-1 were tested for significance within the groups. Using a mixed ANOVA, psychological stressor effects on GH secretion and the possible effects of ERT on this response were evaluated by comparing basal GH to levels of GH measured immediately following the stressors and after an additional 30 min in the three groups. These analyses were carried out using the SPSS Advanced Statistics 6.1 package.

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