Effects of hormone replacement on growth hormone and prolactin exercise responses in postmenopausal women

R. R. Kraemer, L. G. Johnson, R. Halton, G. R. Kraemer, H. Gaines, M. Drapcho, T. Gimple, and V. Daniel Castracane. Effects of hormone replacement on growth hormone and prolactin exercise responses in postmenopausal women. J. Appl. Physiol. 84(2): 703–708, 1998.—Exercise elevates growth hormone (GH) and prolactin (PRL) blood concentrations in premenopausal women. Postmenopausal women taking hormone replacement therapy (HRT) maintain higher estrogen levels that could affect GH and PRL. The purpose of the study was to determine the effects of HRT on GH and PRL responses to treadmill exercise. Seventeen healthy women who were postmenopausal (naturally or surgically) (8 on HRT; 9 not on HRT (NHRT)), completed 30 min of treadmill exercise at 79.16 ± 1.2% maximal O2 consumption (HRT group) and 80.19 ± 0.91% maximal O2 consumption (NHRT group). Blood samples were collected from an intravenous catheter during an exercise session and during a control session without exercise. GH and PRL concentrations were significantly higher in the exercise trial than in the nonexercise trial, whereas resting concentrations were similar for both trials. GH and PRL peaked at 10.8 ± 1.60 and 12.67 ± 2.58 ng/ml, respectively, for HRT subjects and at 4.90 ± 1.18 and 9.04 ± 2.17 ng/ml, respectively, for NHRT subjects. GH concentrations in the exercise trial were significantly higher for HRT than for NHRT subjects. This is the first study to demonstrate that HRT enhances treadmill-exercise-induced GH release and that similar PRL responses to treadmill exercise occur in postmenopausal women regardless of HRT status.

somatotropin; estrogen; menopause

GH has been shown to increase in response to running exercise in eumenorrheic women (17). Low-volume resistive exercise also elicits a GH increase in eumenorrheic women during the luteal phase, but not during the follicular phase, of the menstrual cycle (19). Certain high-volume resistive exercise protocols elicit a GH response as well (21). There are only sparse data concerning GH responses to exercise in postmenopausal women. Exercise may stimulate a greater GH response for women receiving hormone replacement therapy (HRT) than for those not taking estrogen (NHRT). An increased GH response could have a beneficial effect on biological aging. The exercise-induced response may be especially evident for women who take oral estrogen rather than transdermal estrogen. The latter produces a tonic level of estradiol (E2) and does not pass through the liver in high concentrations but distributes E2 directly into the peripheral circulation (1). It has been shown that postmenopausal women receiving oral estrogen have significantly higher 24-h GH concentrations and pulse amplitudes than postmenopausal women taking transdermal estrogen (8, 27); however, treatment with oral and transdermal E2 that results in similar concentrations of circulating E2 increases GH concentrations to the same extent (8).

Prolactin (PRL) and GH share amino acid homologies, and the genes for PRL and GH have similar structure and organization; PRL receptors in humans are also stimulated by GH (28). Both PRL and GH levels have been shown to respond to exercise (6, 14, 17). The lactotrophic cells in the anterior pituitary are increased in size and number by estrogen (28); thus HRT may enhance PRL response to exercise in postmenopausal women. This is the first study to determine whether HRT affects GH and PRL responses to aerobic exercise in postmenopausal women.

METHODS

Subjects. Seventeen women were recruited through newspaper advertisements. The women provided written consent for participation in the study. Eight untrained women were on oral HRT [5 naturally and 3 surgically postmenopausal; mean age 49.38 ± 3.10 (± SE) yr]. Nine untrained women were NHRT subjects (7 naturally and 2 surgically postmenopausal, age 53.0 ± 2.98 yr). The HRT subjects were taking Ogen, Premarin, or Estratest daily. Criteria for participation in the study included 1) being in postmenopausal status, either natural or surgical (removal of both ovaries and uterus); 2) being able to complete 30 min of moderate treadmill exercise; 3) not having chronic illnesses, such as diabetes mellitus, liver or gallbladder disease, coronary heart disease, malignancy, anemia, or renal failure; and 4) not...
taking any medication that could alter hormone concentra-
tions, e.g., β-blockers, glucocorticoids, diuretics, or other
hormonal or hormone-mimetic medications. All subjects were
deemed healthy through medical history screening and a
graded-exercise test with a 12-lead electrocardiogram. Women
were judged to be postmenopausal by 1) surgical removal of
both ovaries and uterus, 2) absence of menses for at least 1 yr,
and 3) baseline gonadotropin levels. The study was approved
by the Southeastern Louisiana University Committee for the
Use of Humans and Animals as Research Subjects and was
completed in accordance with the Declaration of Helsinki.

Nutritional status. To verify that diet was similar for the
HRT and NHRT groups, each subject completed a 3-day food
record during the week before an experimental and a control
trial. By using a computer program (Nutritionist IV-N²
version 3; Salem, OR), food records were analyzed for 1) total
kilocalorie intake, 2) percent of diet that was carbohydrate,
fat, and protein, and 3) saturated and unsaturated fat intake.
Subjects were asked to refrain from exercise or alcohol
ingestion for 48 h before testing.

Session 1. Subjects completed a preexperimental session
for determination of fitness level and to become familiarized
with the treadmill. Skinfold thickness was assessed at four
sites: 1) triceps, 2) abdomen, 3) suprailiac, and 4) thigh (13).
Maximal O₂ uptake (VO₂max) was assessed on a treadmill
with an automated system to measure O₂ consumption (VO₂).
Every 30 s, an expiratory air volume was assessed with a
heated pneumotach (series 3813; Hans Rudolph, Kansas City,
MO) and pressure transducer (VRCD/HC-1; Consentius Tech-
nologies, Sandy, UT), and expired O₂ and CO₂ were analyzed
(S-3A/1 and CD-4 analyzers; Ametek, Pittsburgh, PA). Equip-
ment was interfaced (OUS/MC; Consentius Technologies) to a
personal computer, and values were recorded every 30 s. Before each VO₂max determination, the O₂ and CO₂ gas
analyzers were calibrated with gases of known composition.
The treadmill protocol began at 2 miles/h (mph) and 2%
grade, and every 2 min it was increased by 0.5 mph and 2%
grade until 3.5 mph was reached; thereafter, treadmill grade
only was increased by 3% every 2 min until exhaustion. All
subjects were determined to have reached VO₂max when either
the primary criterion of a plateau in VO₂ with an increase in
workload was met or two of three secondary criteria were
noted: 1) reaching predicted maximal heart rate, 2) respira-
tory exchange ratio >1.0, or 3) a rating of perceived exertion
(10-point Borg Scale) of 9 or 10 (11). Descriptive data are
shown in Table 1. Age, height, weight, body mass index (BMI),
skinfold thickness, and VO₂max of the subjects in the HRT and
NHRT groups were not statistically different.

Sessions 2 and 3. Subjects reported to the exercise physiol-
ology laboratory at 0745 after an overnight fast. A registered
nurse inserted an intravenous catheter (20 gauge, 32 mm;
J elco, Tampa, FL) and attached a normal saline lock. With the
subject in a sitting position, resting blood samples were
collected from the catheter at 0830 (40 min before exercise;
–40) and at 0910 h (10 min before exercise; –10). For each
blood draw, the first 3 ml of blood (with saline from the
catheter lock) was withdrawn into a discard tube preceding
a 25-ml draw. The catheter was then flushed with physiologi-
saline solution (3 ml) to maintain patency. Subjects
completed 30 min of treadmill exercise at 79.16 ± 1.2%
VO₂max (HRT group) and 80.18 ± 0.91% VO₂max (NHRT group).
Percent VO₂max values were not significantly different in NRT
and NHRT groups. Exercise intensity was maintained by ad-
justing speed and grade of the treadmill. Blood was collected
during [after 15 min of exercise (+15)] and immediately after
treadmill exercise [0-min recovery (R0)]. Additional blood
draws were taken in a sitting position at 10, 20, 35, 50, 65,
and 80 min postexercise (R10, R20, and so on). Each subject
completed a second trial that served as a control, and blood
was sampled at the same diurnal times during as the exercise
trial but with the subject resting in a sitting position.

Blood analyses. For each blood draw, samples were col-
collected in two 10-ml whole blood tubes for endocrine determi-
nations, a 5-ml EDTA tube for hematocrit and hemoglobin
assays, and a 3-ml sodium fluoride/potassium oxalate tube for
a colorimetric plasma lactate analysis (Sigma Chemical, St.
Louis, MO). Whole blood was centrifuged, and serum was
divided into aliquots and frozen (−20°C) for subsequent
determination of GH, PRL, luteinizing hormone (LH), follicle-
stimulating hormone (FSH), and E₂. Hematocrit (microcapil-
ary method) and hemoglobin values (Sigma Chemical) were
used to determine plasma volume change (7) and to correct
hormone levels for hemoconcentration shifts that could inher-
ently elevate hormone levels (18). Lactate levels were docu-
mented in the present study to demonstrate the effects of the
exercise on elevated metabolic activity. Baseline concentra-
tions of LH, FSH, and E₂ were determined to verify reproduc-
tive hormone status of the women and to provide a complete
diagnostic profile of each subject.

Lactate and hemoglobin were analyzed spectrophotometri-
cally (Sigma Chemical). Hematocrit was determined by using
a microhematocrit method. GH, PRL, LH, FSH, and E₂ were
analyzed by using an chemiluminescent enzymatic immunoas-
dsay (IMMULITE; Diagnostic Products, Los Angeles, CA). All
hormone serum samples from each subject were determined
in the same assay to avoid interassay variability. Intra-assay
coefficients of variation for GH, PRL, LH, FSH, and E₂ were
all <5.0%. Interassay coefficients of variation for GH, PRL,
LH, FSH, and E₂ were <10%. E₂ interassay coefficients of vari-
ation for middle and high control pools were <10% and for the
low control pool was 14.51%.

Statistics. The data were analyzed by using two different
statistical approaches. First, to examine the total response
of GH and PRL to exercise and estrogen replacement, we
computed an integrated area under the curves (AUC) for GH
and PRL response by using a trapezoidal method after
subtracting averaged baseline hormone concentration for
each subject. AUC is a summary statistic often used in
endocrine research (e.g., Ref. 19) that allows the cumulative
effect of a stimulus on an endocrine parameter to be exam-
ined. Independent t-tests were used to determine whether
hormone concentrations differed between the HRT and NHRT

<table>
<thead>
<tr>
<th>Group</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>BMI, wt/ht²</th>
<th>Sum of Skinfolds, mm</th>
<th>VO₂max, ml·kg⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRT</td>
<td>162.71 ± 1.35</td>
<td>64.72 ± 4.31</td>
<td>24.26 ± 1.03</td>
<td>88.25 ± 9.95</td>
<td>29.37 ± 1.35</td>
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<tr>
<td>NHRT</td>
<td>164.82 ± 1.37</td>
<td>70.40 ± 5.19</td>
<td>25.94 ± 1.98</td>
<td>86.11 ± 11.17</td>
<td>23.79 ± 2.79</td>
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Values are means ± SE for 8 subjects in hormone replacement therapy (HRT) group and 9 subjects in non-HRT (NHRT) group. BMI, body mass index; wt, weight; ht, height; VO₂max, maximal O₂ consumption.
groups. These were one-tailed analyses, as we expected GH and PRL to be higher in the HRT group than in the NHRT group. To ascertain whether AUC for GH and PRL differed between experimental (exercise) and control trials, dependent t-tests were conducted. These were also one-tailed analyses, because we expected GH and PRL to be higher during the exercise trial than during the control trial.

Second, to examine the GH and PRL response to exercise and HRT replacement at specific time points, a 2 × 2 × 10 (trial × group × time point) repeated-measures analysis of variance (ANOVA) was conducted. Significance levels reported reflect Geisser-Greenhouse degrees of freedom adjustments.

Potential confounding variables that could also influence GH and PRL include diet and fitness level. To verify that diet was similar between groups and trials for total kilocalories, percent carbohydrate, percent fat, and percent protein, an independent t-test was conducted. To account for the proportion of GH increase that could be caused by fitness level, we analyzed AUC values by using an analysis of covariance (ANCOVA) with VO\textsubscript{2max} as the covariate. To verify that E\textsubscript{2} and FSH were different between groups, independent t-tests were conducted. These were one-tailed analyses, because we expected E\textsubscript{2} to be higher in the HRT group than in the NHRT group and FSH to be lower in the HRT group than in the NHRT group. The alpha level was set at P < 0.05.

**RESULTS**

Time period −40 and −10 hematocrit and hemoglobin concentrations were within the normal range for resting values. Plasma volume shifts did not exceed 7.4% between any two time points during the exercise trial. Age, height, weight, BMI, VO\textsubscript{2max}, and sum of skinfolds were not significantly different between HRT and NHRT groups. Lactate peaked at 5.79 ± 0.80 mM (+15) for HRT subjects and at 4.60 ± 0.65 mM for NHRT subjects (R0). Resting and peak lactate values were not significantly different between the HRT and NHRT groups during exercise.

GH. GH levels peaked at R0 at 10.8 ± 1.60 and 4.90 ± 1.18 ng/ml for for HRT and NHRT groups, respectively (Fig. 1). The results of t-tests comparing AUC for GH levels in HRT and NHRT groups revealed that both exercise trials were significantly higher than control trials, and AUC for GH levels in the HRT group was significantly higher than that for NHRT in the exercise trial (Table 2).

The repeated-measures ANOVA revealed a significant main effect for trial [F(1, 30) = 16.82; P < 0.01] and group [F(1, 30) = 5.82; P < 0.05], as well as for several significant interactions, including trial × group × time [F(9, 270) = 2.85; P < 0.05]. Further investigation of this interaction by using the Newman-Keuls test revealed significant differences between groups were localized at four time points: +15, R0, R10, and R20. At these times, GH levels were higher during the exercise trial than during the control trial for both HRT and NHRT groups, and the concentrations during exercise were higher for the HRT group than for the NHRT group (Fig. 1).

PRL. PRL peaked at 12.67 ± 2.58 ng/ml (R0) for HRT subjects and at 9.04 ± 2.17 ng/ml (R10) for the NHRT group (Fig. 2). The t-test results indicated AUC values

**Table 2. Area-under-the-curve concentrations for growth hormone and prolactin**

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<th>AUC HRT</th>
<th>AUC NHRT</th>
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<td></td>
<td>Exercise</td>
<td>Control</td>
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<tr>
<td>Growth hormone, ng/ml</td>
<td>290.37 ± 70.39†</td>
<td>−96.40 ± 55.60</td>
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<td>Prolactin, ng/ml</td>
<td>237.12 ± 138.51*</td>
<td>−203.14 ± 62.01</td>
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<td>Growth hormone mean adjusted for VO\textsubscript{2max}, ng/ml</td>
<td>286.72 ± 70.39</td>
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Values are means ± SE for 8 subjects in HRT group and 9 subjects in NHRT group. AUC, area under the curve. *P < 0.05 vs. control; †P < 0.05 vs. NHRT.
for PRL in the HRT and NHRT groups during exercise were significantly higher than those in control trials, but not significantly different from each other (Table 2).

The ANOVA comparing PRL concentrations at different time points revealed a significant main effect for trial [F(1, 28) = 4.26; P < 0.05] and time [F(9, 252) = 4.03; P < 0.01]. However, both main effects were superseded by a significant trial × time interaction [F(9, 252) = 6.18; P < 0.01]. A Newman-Keuls test was used to further investigate these findings; it revealed that PRL was significantly higher during the exercise trial than during the control trial at R0, R10, R20, R35, and R50.

Potential confounding variables. Mean FSH, LH, and E2 concentrations were within the normal range, as established by our laboratory, for postmenopausal women on HRT and NHRT (Table 3). As expected, E2 was significantly higher in the HRT group, and FSH was significantly higher in the NHRT group. One potential variable influencing GH responses to exercise is fitness level of the subjects. The results of an ANCOVA, using VO2max as the covariate and AUC for GH as the dependent measure, revealed a significantly higher AUC for GH for the HRT group [F(1, 15) = 6.16; P < 0.05]. Adjusted means for GH are shown in Table 2. Moreover, we evaluated the relationship between VO2max and the magnitude of the exercise-induced change in GH concentrations (AUC) by using a Pearson correlation coefficient, which revealed a small, nonsignificant correlation (r = 0.27, P > 0.10).

Nutritional data were analyzed for daily total kilocalorie intake, percent of total kilocalories that were carbohydrate, percent of total kilocalories that were fat, and polyunsaturated/saturated fat ratio. Comparisons by t-test indicated that none of the nutritional data was significantly different between experimental and control trials for each group nor experimental trials between HRT and NHRT groups (Table 4).

DISCUSSION

We have demonstrated that GH responses to treadmill exercise are higher in postmenopausal women receiving HRT than in those not on HRT. However, in response to treadmill exercise, PRL increases in postmenopausal women regardless of their estrogen status. Our research design controlled for GH pulsatility by using a control trial and short intervals between blood sampling. This is the first study to document effects of HRT in postmenopausal women on their GH and PRL responses to a well-quantified exercise bout.

Circulating GH and IGF-I concentrations have been shown to decline with age in women of reproductive age and postmenopausal women (27). The age-related decline in GH pulses may be explained partially by an increase in somatostatin tone, because the administration of agents that inhibit somatostatin release, i.e., cholinergic agonists and arginine, have been demonstrated to increase GH release in older persons (23, 29). There is evidence that GH and IGF-I facilitate anabolic functions of bone formation and protein synthesis in muscle (2). The data from our investigation underscore the importance of incorporating exercise into the lifestyle of postmenopausal women, whether on or off estrogen replacement, to reduce catabolic effects associated with aging.

Evidence suggests that oral estrogen administration to postmenopausal women elicits an increase in GH-pulse height, individual GH-pulse amplitude, and incremental GH-pulse amplitude (8, 26). Although both oral and transdermal HRT have been shown to increase blood levels of GH, neither has been shown to com-

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<th>Table 3. Baseline (−10) growth hormone, prolactin, gonadotropin, and estradiol concentrations</th>
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<td>HRT</td>
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Values are means ± SE for 8 HRT subjects and 9 NHRT subjects. GH, growth hormone; PRL, prolactin; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, estradiol.

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<th>Table 4. Average daily kilocalorie intake, carbohydrate intake, fat intake, protein intake, and polyunsaturated/saturated fat ratio</th>
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Values are means ± SE for 8 subjects in HRT group and 9 subjects in NHRT group. Nos. in parentheses, %total intake.
pletely reverse age-related reductions (2). Mechanisms suggested for the E₂ effect on increased GH include the expression of E₂ and somatostatin receptors in the hypothalamus by GH-releasing hormone neurons (24) and reduced IGF-I negative feedback effect on GH secretion (1–8). We have previously shown that exercise-induced increases in GH do not result in short-term increases in IGF-I concentrations (20); therefore, we did not measure IGF-I values in the present study.

Dawson-Hughes et al. (5) reported a slightly higher GH response to light exercise in postmenopausal women treated for 2 wk with ethinyl E₂. The exercise intensity was not well quantified (walking at ~1 mph as determined by a pedometer) and appeared to be considerably lower than that used in the present study. It has been shown that the mode and intensity of exercise affect the GH response (17–19, 21). We have previously documented elevated GH concentrations in young women in response to treadmill running (17) and to resistive exercise during the luteal phase of the menstrual cycle (19). One mechanism explaining enhanced exercise-induced increases in GH increases via E₂ includes increased pituitary sensitization leading to increased release of GH-releasing hormone (19), which could explain the observations from the present study. Another mechanism, hemoconcentration, was ruled out as an explanation for increased GH and PRL levels in the present study, because peak percent increases in GH and PRL were well above the greatest percent PV reduction that would have caused increased hormonal concentrations.

It has been shown that PRL secretion is increased by E₂ through 1) increasing baseline secretion, 2) increasing release of thyroid-releasing hormone, and 3) reducing the effects of dopamine agonists on inhibition of PRL secretion (25). PRL responses to exercise have been observed in eumenorrheic runners, but the PRL responses were absent in amenorrheic runners (22), and those findings were suggested to be caused by extreme ovarian suppression. Our data, however, suggest that treadmill exercise at ~80% of VO₂max increases PRL concentrations in postmenopausal women, regardless of their HRT status.

Keizer et al. (16) showed that PRL rises in trained and untrained eumenorrheic women when they are exercised to exhaustion (above lactate threshold). Because lactate levels were increased during exercise in the present study, it would appear that lactate threshold was exceeded and produced a PRL response even in the NHRT postmenopausal women.

For a number of reasons, we do not think there was a difference in physical exertion between the HRT and NHRT groups. First, the percentages of VO₂max maintained by the NHRT and HRT groups were similar. Second, lactate concentrations in the HRT and NHRT groups were not significantly different at rest or peak exercise. Third, the rating of perceived exertion reported for the NHRT group was similar to that of the HRT group during exercise (5.10 for HRT group vs. 5.30 for NHRT group on the 10-point Borg scale). Fourth, the percent of maximum heart rate maintained by both groups during exercise was not significantly different (90.23 vs. 89.49% for HRT vs. NHRT group, respectively; P > 0.05).

It has previously been shown that higher fitness levels are associated with greater GH response to exercise (3). Not only was VO₂max similar between groups (P > 0.05), but even with the variance accounted for by VO₂max removed in an ANCOVA analysis, the GH concentrations were still significantly and substantially higher in the HRT group compared with the NHRT group. Moreover, no significant relationship between fitness level and GH response (AUC) was found.

Diet may also affect GH concentrations (12); however, it is unlikely that diet affected the results of the present study. Food records indicated that the diets of subjects in the HRT and NHRT groups were not significantly different in total caloric intake as well as in percent carbohydrate, fat, and protein; moreover, the nutrient composition and caloric values were close to recommended daily allowance values.

The exercise response of other hormones may also be affected by HRT. For example, we have recently documented an enhanced exercise response of the adrenal hormones dehydroepiandrosterone and cortisol in the same subjects who participated in the present study (15). Future investigations are needed to examine exercise responses and adaptations of hormones affected by E₂.

In summary, this is the first study to demonstrate an augmented response of GH to treadmill exercise by postmenopausal women on HRT and similar PRL responses in postmenopausal women regardless of HRT or NHRT status. The effect of HRT and exercise may serve to enhance the obvious health benefits produced by HRT or by exercise alone.

The authors are grateful to the women who participated as subjects in this study. We especially thank Dr. Edward P. Hebert for advice and help with statistical analyses. We also wish to thank Diagnostic Products Corporation, Los Angeles, CA, for their contribution of radioimmunoassay materials. This study was made possible by a faculty development grant from Southeastern Louisiana University.

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