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TITLE

GROWTH HORMONE RESPONSES TO CONTINUOUS AND INTERMITTENT EXERCISE IN FEMALES UNDER ORAL CONTRACEPTIVE THERAPY

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ORIGINAL ARTICLE

R.P. Bernardes · M.W. Radomski

Growth hormone responses to continuous and intermittent exercise in females under oral contraceptive therapy

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Abstract In this study we investigated the effect of oral contraceptive (OC) use (OCU) and non-use (OCNU) on growth hormone (GH) responses to exercise in the same females (n = 7, age 22–31 years) during the normal course of OC therapy. Continuous (60% maximum oxygen consumption, $\dot{V}O_{2max}$ for 20 min) and intermittent exercise (>80% $\dot{V}O_{2max}$) protocols of equal total duration, and similar external work were performed during phases of OCNU (days 3–5 of the menstrual cycle) and OCU (days 7–11). Levels of GH, lactate, 17 β-estradiol, and progesterone were measured. Lactate responses were significantly greater ($P < 0.05$) during intermittent than continuous exercise, with no effect of OC use. However, significantly greater GH responses were found during the OCU phase than the OCNU phase in both the continuous (+94%) and intermittent (+250%) exercise protocols. Estradiol and progesterone levels increased significantly during exercise in all four conditions. We suggest that the increased GH responses observed during the OCU-phase were potentiated by the elevated levels levels of total estrogens (endogenous 17 β-estradiol and exogenous ethinyl estradiol). It is suggested that training programs for female athletes could be timed in accordance with the menstrual cycle to benefit from an increased GH response to exercise during phases of OC use or the luteal phase of women not on OC therapy.

Key words Growth hormone · Oral contraceptives · Estradiol · Progesterone · Intermittent and continuous exercise

R.P. Bernardes
School of Physical Health and Education,
University of Toronto, Toronto,
Ontario, Canada M5S 1A1

M.W. Radomski (✉)
Defence and Civil Institute of Environmental Medicine,
North York, Ontario, Canada, M3M 3B9

Introduction

A variety of factors influence the release of growth hormone (GH) during exercise in males, including the type, intensity and duration of the exercise, and the fitness levels of the subjects (Bunt et al. 1991; Karagiorgos et al. 1979; Kinderman 1982; Kraemer et al. 1990; VanHelder et al. 1984). However, relatively little research has been done on females, partly because of the confounding variables associated with the menstrual cycle (Bonen et al. 1991; Hansen and Weeke 1974; Kanaley et al. 1992). Although resting females have higher plasma levels of GH than resting males, a possible result of the potentiating effect of estrogen on GH secretion (Frantz and Rabkin 1965; Ho et al. 1987; Kraemer et al. 1991), it is unclear whether the menstrual phase, with its fluctuating estrogen levels, affects the GH responses to exercise.

GH responses to moderate exercise have been reported to be significantly greater in females during the mid-cycle of the menstrual period when estrogen levels are elevated (Hansen and Weeke 1974). Conversely, other studies have reported that in female runners the incremental GH responses to exercise are not influenced by menstrual phase (Kanaley et al. 1992). These differences in the literature may be partially attributable to differences in exercise protocols, subject selection, and large intersubject variability in GH responses (Raynaud et al. 1983).

Females under oral contraceptive (OC) therapy offer an excellent opportunity to study the effects of the menstrual phase on the GH responses to exercise because OCs regulate the menstrual cycle and stabilize the levels of estrogen, progesterone and reproductive hormones. In the present study we examined the GH responses in young females under OC therapy to continuous and intermittent exercise protocols, standardized for equal total duration and total external work. The exercises were performed during the phases of OC non-use (OCNU, days 3–5 of the menstrual cycle)

and OC use (OCU, days 7–11 of the menstrual cycle). We hypothesized that there would be an increased GH response to exercise mediated by the elevated levels of exogenous estrogen during the OCU phase compared to the OCNU phase.

Methods

Subjects

Seven healthy nulliparous females, aged 22–31 years initially volunteered as subjects for the study after giving their informed consent. They were medically examined and found to be free of any endocrine disorders. The subjects were not ingesting any pharmaceuticals apart from OC therapy. All subjects had experienced menarche at least 5 years prior to the commencement of the study, had been eumenorrheic, as defined by a normal 28-day menstrual cycle over the previous year, and had been continuous users of OCs for at least 1 year. The OC used by each subject and its chemical formula are shown in Table 1. The synthetic estrogenic component, ethinyl estradiol, was common to all of the OC preparations, with less than a 5-μg difference in the amount of ethinyl estradiol between different preparations.

The subjects' physical characteristics were: [mean (SEM)] age, 26 (1.1) years; body mass, 61.3 (2.5) kg; height, 168 (1.8) cm; maximal aerobic power ($\dot{V}O_{2max}$), 45.9 (1.4) ml · kg^{-1} · min^{-1}, and body fat, 19.2 (1.4)%. Body fat was estimated by using the sum of five skinfold thickness measurements (Katch and Katch 1984). $\dot{V}O_{2max}$ was determined using the cycle ergometer test outlined by Astrand and Rodahl (1977).

Each subject performed a graded exercise protocol which consisted of continuous cycling at four intensities (30%, 50%, 70% and 80%) of $\dot{V}O_{2max}$ for 4 min or until oxygen consumption plateaued. An exercise intensity equivalent to 60% of each subject's $\dot{V}O_{2max}$ was calculated based on the linear regression of Watts versus oxygen consumption.

Experimental design

The experiment was a 2 × 2 design and consisted of continuous and intermittent cycling exercise protocols performed during phases of OCU (days 7–11 of the menstrual cycle) and OCNU (days 3–5). The four experimental trials were conducted in a randomized fashion. Each trial was separated by at least 1 week, and the four protocols were performed by each subject over a period of 2–3 menstrual cycles. Before each of the trials, subjects were asked to fast overnight and refrain from sexual activity, strenuous physical exercise, and ingestion of alcohol or caffeine for the 24 h prior to testing. Subjects were always tested in the morning (0700–1000 hours) to standardize the effects of circadian variations in hormone concentrations.

The OC administration schedule consisted of 21 days of OC ingestion (OCU) followed by a non-use period of 7 days (OCNU) of placebo ingestion. Testing during the OCNU phase occurred on days 3–5 of the menstrual cycle when the subjects abstained from OCU in the normal course of therapy. In normally menstruating females not under OC therapy, this is a period of low endogenous estrogen levels, as levels gradually peak towards mid-cycle (Keizer and Rogol 1990). Although full metabolic clearance of exogenous estrogen from the circulation after the last day of OC ingestion of the previous menstrual cycle was unlikely, significant levels of it were expected to have cleared (Bonen et al. 1991). Testing during the OCU phase occurred during days 7–11 of the menstrual cycle when total estrogen levels were expected to be higher than in the OCNU phase. It is unlikely that production of endogenous estrogen had been fully suppressed given that OC ingestion started on day 6 (Kuhl et al. 1985).

Exercise protocols

During the continuous exercise protocols subjects cycled at an exercise intensity equivalent to 60% of their $\dot{V}O_{2max}$ for 20 min. The mean (SEM) exercise intensity performed was 102.4 (9.0) W and the total mean (SEM) external work was 122.9 (10.8) kJ.

In the intermittent exercise protocol, subjects cycled at an intensity equal to or greater than 80% of their $\dot{V}O_{2max}$ for 75 s, followed by a 50-s rest interval. This cycle was repeated ten times in a 20-min time period for a total of 12.5 min of cycling and 7.5 min of rest. The mean (SEM) exercise intensity performed during the intermittent exercise periods was 164.0 (14.4) W. The total mean (SEM) external work was 123.0 (10.8) kJ. There was no significant difference in the total external work performed between the continuous and intermittent protocols. For all trials, subjects remained seated on the ergometer during the recovery period until blood sampling had been completed.

Blood collection and biochemical analyses

In each trial, an indwelling intravenous catheter was inserted into an antecubital vein 35 min prior to the start of exercise. Serial blood samples (13 ml) were taken at −10, 0, 4, 8, 12, 16, 20, 30, 40, 60, and 90 min with respect to the beginning of exercise (total volume of 143 ml). The intravenous catheter was kept patent by the infusion of 0.6 ml heparin-saline solution (100 units · ml^{-1}) after each sampling.

Samples for GH, estrogen and progesterone analysis were collected into two 5-ml vacutainer tubes containing 0.05 ml of ethylenediamine-N,N,N,'N'-tetra acetic acid (15%) solution. Immediately after sampling, the contents were mixed by gentle repeated inversion and then immersed in an ice-water (4°C) bath until centrifugation. Blood samples for lactate analysis were collected into vacutainer tubes containing sodium fluoride (7.5 mg) and potassium oxalate (6.0 mg). A 25-μl aliquot of blood was then mixed vigorously with 200 μl of 0.4 M perchloric acid, and immediately stored at −70°C. The remainder of the blood sample

Table 1 Type and formulation of oral contraceptives (*OC*) used by subjects

OC Type	Subject number	Formula progesterone + estrogen
Wyeth Triphasil 21	1, 2, 3	6 × (50 μg levonorgestrel + 30 μg ethinyl estradiol) 5 × (75 μg levonorgestrel + 40 μg ethinyl estradiol) 10 × (125 μg levonorgestrel + 30 μg ethinyl estradiol)
Ortho 7/7/7	4, 5	7 × (0.5 mg norethindrone + 35 μg ethinyl estradiol) 7 × (0.75 mg norethindrone + 35 μg ethinyl estradiol) 7 × (1 mg norethindrone + 35 μg ethinyl estradiol) 7 × lactose
Ortho 1/35	6, 7	1 mg norethindrone acetate + 35 μg ethinyl estradiol + lactose

was centrifuged for 15 min at 1500 g and the separated plasma immediately frozen and stored at −70°C until analysis.

Hormone concentrations were determined using commercially available, standard double-antibody radioimmunoassay (RIA) kits: GH using the Nichols Institute Diagnostics RIA (assay sensitivity of 0.02 ng · ml^{-1} and intra- and inter-assay coefficients of variation of 4.2% and 7.2%, respectively); progesterone by the Binax Equate RIA kit (sensitivity of 0.04 ng · ml^{-1} and intra- and inter-assay coefficients of variance 7.3% and 8.6% respectively); and estrogen (17 β-estradiol) by the Pantex Direct Estradiol kit (assay sensitivity 10 pg · ml^{-1} and intra- and inter-assay coefficients of variation 4.7% and 7.1%, respectively). Lactate concentrations were determined by Maughan's technique (1982). Duplicate analyses with a coefficient of variability greater than 10% were re-analyzed. The total incremental GH response to each exercise was determined by calculating the area under the GH response curve (AUC) from the start of exercise (t = 0 min) to the approximate return of all values to initial resting values (t = 40 min), using the trapezium rule (Mathews et al. 1990).

Statistical analysis

An analysis of variance (ANOVA) with repeated measures was used to compare the responses of the two exercise groups (continuous and intermittent), the effects of the two phases (OCNU and OCU), and the responses during the exercise and post-exercise time period. Since the values of a given measure at different levels of the repeated factor are not independent, but are highly correlated, the Greenhouse-Geisser correction was applied to these ANOVAs to reduce the risk of a Type 1 error. All data are given as the mean (SEM).

Results

Lactate responses

Lactate levels were significantly elevated from the first exercise sampling time (t = 4 min) until 20 min of recovery ($P < 0.05$) in both the OCU and OCNU phases of continuous and intermittent exercise (Fig. 1). Intermittent exercise elicited significantly ($P < 0.05$) greater increases in lactate levels than continuous exercise, but there were no significant differences between the OCU and OCNU phases within each exercise protocol.

GH responses

No significant differences in the resting plasma GH levels were found between the OCU and OCNU phases. Figure 2 shows the mean changes in plasma GH in response to the continuous and intermittent exercises performed during the OCU and OCNU phases. Plasma GH levels increased significantly from resting levels for both exercise conditions and OC phases ($P < 0.05$), peaking at the end of the exercise period. In both exercise protocols, the GH responses were significantly higher ($P < 0.01$) during the OCU phase than the OCNU phase (t = 10–30 min).

The type of exercise (continuous versus intermittent) had no significant effect on the total incremental GH responses (AUC) during the OCNU or OCU phases (Fig. 3A, B). However, a significantly greater total GH response ($P < 0.05$) occurred in the OCU phase than in the OCNU-phase, independent of the type of exercise (Fig. 3C, D).

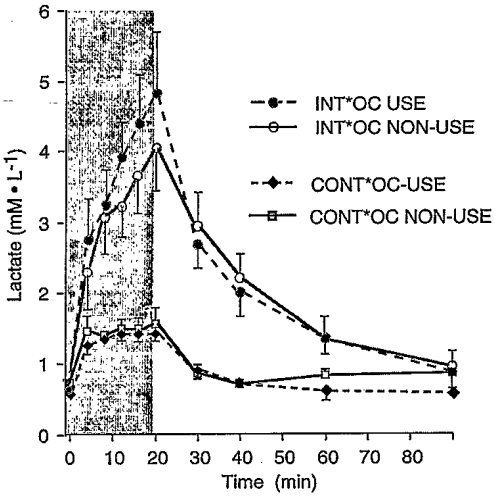

Fig. 1 Blood lactate responses (mean ± SEM) during continuous (*CONT*; *diamonds* and squares) and intermittent (*INT*; *circles*) exercises with (*dashed lines*) and without (*solid lines*) oral contraceptive (*OC*) use. The *shaded area* indicates the period of exercise. Values for intermittent exercise are significantly higher ($P < 0.01$) than for continuous exercise from t = 4 to t = 20 min

Estradiol and progesterone responses

The mean 17 β-estradiol responses for the four experimental conditions are shown in Fig. 4A. Lower circulating levels of endogenous 17 β-estradiol were found in the OCU phase than the OCNU phase throughout the exercise and recovery period, a result of the inhibitory effect of OC therapy on endogenous estrogen production.

Endogenous estradiol levels increased significantly from resting values throughout the exercise period and peaked after 10 min of the termination of exercise, followed by a gradual decrease to baseline values by 60-90 min. A main effect ($P < 0.05$) of exercise was evident from t = 4–40 min. ANOVA also showed an OC phase effect, with a greater endogenous estradiol response occurring during the OCNU condition than the OCU condition ($P < 0.05$; Fig. 4A).

Progesterone levels increased during exercise in all conditions (Fig. 4B) and peaked at the termination of exercise, returning to pre-exercise values by 60 min. The ANOVA revealed a main effect of time ($P < 0.05$), but there were no significant effects of phase or exercise type on the progesterone responses to exercise.

Discussion

In the present study we examined the GH responses to exercise in female subjects under normal OC therapy during menstrual cycles that were consistent in terms of length and stability. Every effort was made to normalize as many factors as possible given the effect of circadian rhythms and the high inter-individual variability inherent in GH levels (Raynaud et al. 1983). Since estrogen is

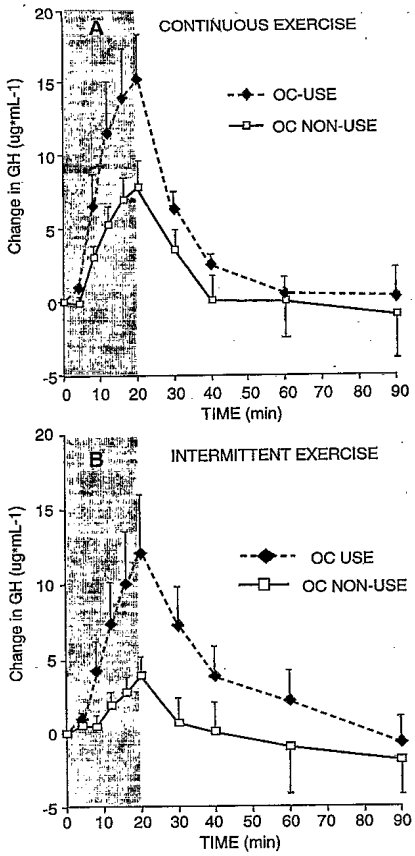

Fig. 2A, B Changes in growth hormone (*GH*) levels from resting levels (mean ± SEM) in continuous (**A**) and intermittent (**B**) exercise during OC use and OC non-use. The GH levels following 20 min of exercise were significantly ($P < 0.01$) higher in the OC use than the OC non-use phase in both exercise protocols. The *shaded area* indicates the period of exercise

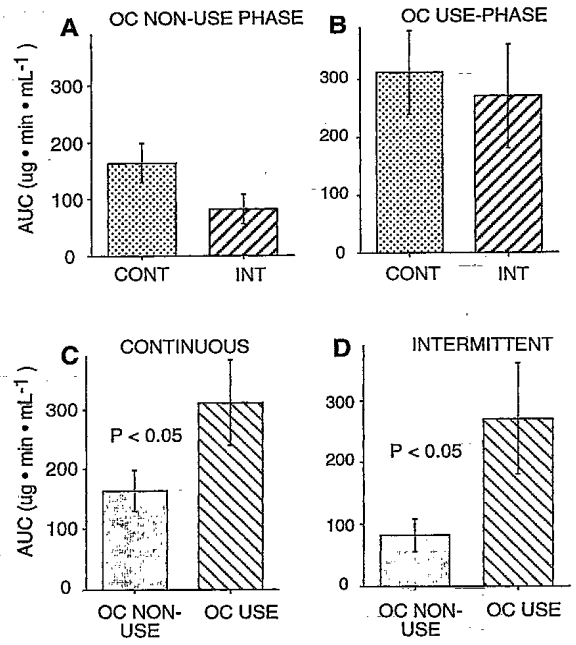

Fig. 3A–D Total GH responses during exercise and recovery represented as the area under the curve for: **A** OC non-use and **B** OC use for continuous (*CONT*) and intermittent (*INT*) exercise; **C** continuous (*CONT*) and **D** intermittent (*INT*) exercise for OC non-use and OC use. Values are means ± SEM

known to stimulate GH secretion (Frantz and Rabkin 1965; Ho et al. 1987), we hypothesized that a heightened GH response to exercise would occur during the OCU phase, when plasma levels of total estradiol (endogenous plus exogenous) would be elevated. The greater exercise-induced increases in GH secretion found during the OCU phase than in the OCNU phase confirmed our hypothesis.

Reports on GH levels in normally menstruating women have provided conflicting results. Some researchers have described a distinct GH peak around ovulation in resting subjects (Frantz and Rabkin 1965; Yen et al. 1970), while others have found similar fasting serum GH levels at menstruation and mid-cycle (Hansen and Weeke 1974; Merimee et al. 1969). Our results concur with the latter findings in that no significant differences in resting GH levels were found in either OC phase. During exercise, increased GH responses have been found at mid-cycle (Hansen and Weeke 1974), but others are not in agreement (Kanaley et al. 1992). The latter study used aerobically trained female athletes as subjects, and the training adaptations in these subjects may have out-weighed phase differences in GH responses.

Our study reports a statistically greater GH response during exercise and recovery in the OCU phase (days 7–11 of the menstrual cycle) compared to the OCNU phase (days 3–5). Previous investigations of women under OC therapy have reported no phase effect on the GH response to exercise (Bonen et al. 1991; Hansen and Weeke 1974). This discrepancy may be due to differences in the standardization of factors that influence the GH response to exercise. In the former study (Bonen et al. 1991), the exercise protocol consisted of 30 min of mild treadmill exercise followed by 30 min of heavy exercise. The mild exercise was of a very low intensity and did not elicit a GH response above resting levels during the OCU -phase. Although Bonen et al. (1991) reported higher levels of GH during mild exercise in females using OCs compared to normal women in the luteal phase, this was a result of higher resting levels of GH in their females under OC therapy and not due to the exercise. The levels of GH observed during their mild exercise were similar to the resting levels in their subjects. Hansen and Weeke (1974) employed an exercise protocol (20 min of cycling at a moderate intensity) which may not have been of sufficient intensity to elicit a phase effect. Bemben et al. (1992) reported significantly higher GH responses during a 90-min treadmill exercise at 50% $\dot{V}O_{2\max}$ in OC users compared to normal women during the early part of their exercise, but found no differences in the total GH responses (AUC) between their groups.

It has been established that sex steroids are markedly increased during exercise in normally menstruating

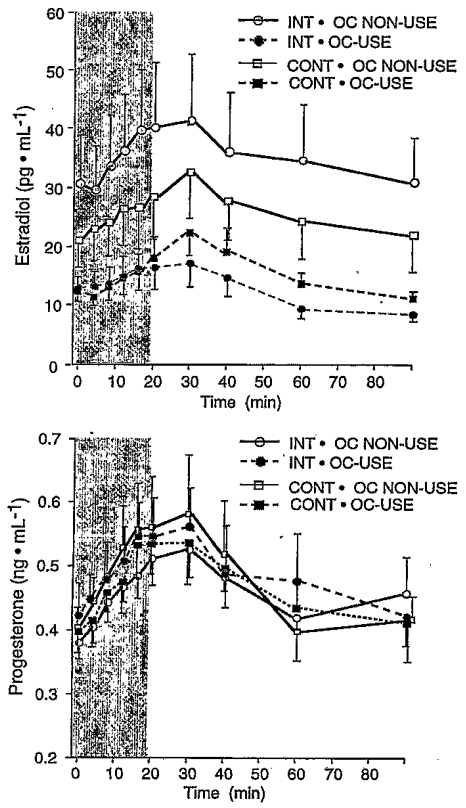

Fig. 4A, B Plasma 17 β-estradiol (**A**) and progesterone (**B**) responses (mean ± SEM) during continuous (*CONT, Squares*) and intermittent (*INT, circles*) exercise during OC use (*dashed lines*) and OCN non-use (*solid lines*). The *shaded area* indicates the period of exercise. Levels following 20 min of exercise were significantly higher than resting levels ($P < 0.05$) in all four conditions

women (Bonen et al. 1983; Jurkowski et al. 1978). However, an investigation of women using OCs revealed no increases in progesterone concentrations (Bonen et al. 1991). This may have been due to the low intensity of the exercise performed in that study since we found significant increases in progesterone levels during exercise that were not associated with either the OC phase or exercise type. We did not observe any relationship between progesterone and GH or OC use during exercise.

Estrogen is known to augment basal GH levels in both males and females (Duursma et al. 1986; Karlsson et al. 1990; Wiedemann et al. 1976), but it is interesting to note that women have higher resting GH levels than men only during waking, ambulatory periods. During sleep periods, both sexes have similar GH levels (Frantz and Rabkin 1965) and it has been suggested that the higher waking ambulatory GH levels in females are not only due to estrogen but may also be mediated by physical activity.

In the present study, the exercise-induced increases in endogenous estradiol (17 β-estradiol) levels showed a phase effect with higher 17 β-estradiol levels in the OCNU condition than the OCU condition ($P < 0.05$). One explanation for the apparently higher estradiol responses to exercise during the OCNU phase may be found in the methods used to assay estradiol levels. Current radioimmunoassays measure only endogenous estrogen, 17 β-estradiol, which is the primary estrogenic hormone in the body. The synthetic estrogen present in OCs is ethinyl estradiol which, although biologically active, is not immunologically active and therefore undetected by commercially available radioimmunoassays. Our radioimmunoassay technique was only able to measure levels of the endogenous 17 β-estradiol and not ethinyl estradiol. We are not aware of any relevant study that has been able to measure levels of both endogenous and exogenous estradiol.

During the OCNU phase, subjects did not ingest any estrogenic compounds so that the only estrogen in their system was from their own small endogenous production of 17 β-estradiol, whereas the total circulating levels of estradiol in the OCU phase consisted of both the endogenous and exogenous estradiol. It is unlikely that any exogenous ethinyl estradiol from the OCU cycle carried over into the OCNU phase, since it was expected to have been almost totally metabolically cleared before exercise in the OCNU phase (Bonen et al. 1991). Thus, it is likely that the significantly greater GH levels found during exercise and recovery in the OCU phase were potentiated by the elevated levels of exogenous synthetic ethinyl estradiol.

Physical training implications

It is known that in healthy normal males, exercise-induced muscle hypertrophy may, in part, result from increased GH secretion (Crist et al. 1988; Florini 1987). Furthermore, short-term GH treatment of adults with GH dificiency has resulted in improvements in muscle strength (Rutherford et al. 1995), increased exercise capacity, and normalization of body composition (Christiensen and Jorgensen 1991). Based on our findings, females on OC therapy may best be able to adapt to the greatest training intensity during the OCU phase when total estrogen levels are at their highest and the subsequent GH responses to exercise maximal.

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