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The Prolactin Response of Males to a Standard MVO_2 Treadmill Test

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The concentration of serum prolactin in response to a standard MVO_2 treadmill stress test to self-perceived exhaustion was investigated in 19 normal healthy males. The prolactin concentration remained stable for an interval of approximately 20 minutes from the beginning of exercise. Peak prolactin levels, observed after the subjects had stopped exercising, indicated a mean 2.6-fold increase over pre-exercise levels. The concentration of prolactin did not increase before an exercise intensity reflecting a VO_2 of 40 ml/kg/min had been reached. There was no relationship between the MVO_2 of the 19 male subjects and their prolactin increment in response to exercise.

Key words: prolactin, exercise, MVO_2, VO_2, increment.

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The influence of prolactin (PRL) on human reproduction is becoming more evident through studies of PRL dynamics. Hyperprolactinemic females may develop galactorrhea, menstrual irregularities, or delayed puberty, while males with hyperprolactinemia may be impotent, hypogonadal or infertile (Boyd and Reichlin, 1978; Frantz, 1978; Yen, 1978; Horrobin, 1979; Ferland et al, 1980; Tolis, 1980; Shangold et al, 1981).

Several studies have shown that exercise is associated with a significant rise in PRL levels in healthy men and women (Noel et al, 1972; Aakvaag et al, 1978; Hagen and Galbo, 1979; Brisson et al, 1980, 1981; Cohen et al, 1980; Mayer et al, 1980; Johannessen et al, 1981; Moretti et al, 1981, 1982, 1983; Prior et al, 1981; Shangold et al, 1981; Jezova et al, 1982, 1983; Nguyen et al, 1982; Bazzarre and Royster, 1984). Although much has been reported about the relationships between exercise, PRL, and reproductive disorders in women, considerably less has been written about these associations in men. The increasing documentation of sports-related infertility syndromes in both men and women (Frisch et al, 1976; Dale et al, 1979; Shangold et al, 1981; Wheeler et al, 1984; Frisch, 1985; Wall and Cumming, 1985), suggests that a study of the nature of the PRL response to exercise in men would be important.

The present study was designed to examine the PRL responses of men exercising to self-perceived exhaustion during a standard MVO_2 treadmill stress test.

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Materials and Methods

Men Studied

Nineteen healthy, male volunteers, 20 to 29 years of age, were tested in the Kinanthropology Laboratory of the University of Ottawa. Their mean height was 5'8" (range 5'5" to 6'3"); mean weight was 170 pounds (range 149 to 227 pounds). All men were required to be healthy and to undergo a practice round simulating the treadmill test before providing their informed consent. The protocol was judged to be ethically acceptable by the University of Ottawa Ethics Committee.

Experimental Protocol

The experimental protocol consisted of a 45-minute preexercise rest period, the treadmill MVO_2 test and a 30-minute recovery period. VO_2 is a measure of the rate of oxygen consumption during exercise. It is expressed as ml oxygen consumed/kg/min. MVO_2 is a measurement of the maximum rate at which an individual can consume oxygen.

Preexercise Period: Individual testing sessions were begun at either 8:30 A.M. or 10:30 A.M., following a 12-hour fast. Three blood samples for PRL were obtained at 15-minute intervals, commencing 15 minutes after establishing an infusion of normal saline in a forearm vein, run to keep the vein open. The mean of the final two PRL levels was used as the "preexercise PRL concentration" when assessing the response to exercise. Blood samples for lactate and hematocrit determination were obtained at the same time as the final preexercise PRL sample. Heart rate monitoring was begun 5 minutes prior to starting the treadmill test (Avionics Stress Test Monitor, Cardioguard Model 2900B, Montreal, Canada).

MVO_2 Treadmill Stress Test: The treadmill protocol was a medium-high intensity stress test (Jette, 1979) using a Quinton treadmill with a Quinton Instruments treadmill control (Seattle, WA). The protocol provided for Stages 1 through 14, each stage lasting 2 minutes with speeds of 3 to 3.75 mph on a grade of 0 to 26%. The workload was increased at each stage by a change in speed or grade.

The subjects were instructed to walk on the treadmill for as long as possible and to signal when no longer able to continue. Expired gases were collected during the final 30 seconds of every second stage, that is, every 4th minute, up to the 10th stage. Thereafter, they were collected in the final 30 seconds of every stage and when the subject signalled exhaustion.

Gases were analyzed by a Godart oxygen analyzer and Godart Capnograph CO_2 analyzer (Stratam-B.V., Bilthoven, Holland). Blood samples for PRL determination were obtained in the final 30 seconds of every 2nd stage, without disturbing the treadmill walk, and when the subject signalled exhaustion.

Recovery Period: The immediate postexercise period allowed for a gradual lowering of the treadmill to 0% grade and a cool down walk at 1.5 mph for up to 2 minutes. The men sat for the rest of the recovery period, during which heart rate was measured and serum samples for PRL were obtained at 6, 12, 18, 24, and 30 minutes. Blood samples for lactate and hematocrit determination were collected at the 6th postexercise minute.

The blood samples for PRL determination were allowed to clot at room temperature and then centrifuged at 1000 × g for 10 minutes. The serum was frozen at −20 C until analyzed. PRL was measured in duplicate by a specific radioassay kit supplied by the Amersham Corp. (Arlington Heights, IL) as described previously (Hudson et al, 1986). The intraassay coefficient of variation was 5.7% for PRL values between 10 and 30 ng/ml and 13% for PRL values below 10 ng/ml. The interassay coefficient of variation was 5.4%. All serum specimens for each man were analyzed during the same assay. With this assay, the normal range for men is 2 to 10 ng/ml.

Blood samples for lactate and hematocrit determination were collected in a heparinized vacutainer tube. An aliquot of blood was deproteinized with 1 ml perchloric acid (0.6 M), centrifuged at 1000 × g for 10 minutes and the supernatant was stored at −20 C until assayed for lactate. The remaining blood sample was used for hematocrit determination. Lactate was analyzed spectrophotometrically by a Boehringer-Mannheim GmbH Diagnostic Test Kit (Dorval, Canada).

For each individual, a significant change in PRL level is defined as a peak PRL concentration more than 3 standard deviations higher than the mean of the preexercise serum concentrations. The Sign test was used to determine the statistical significance of the number of subjects meeting this criterion. Students t-test was used to determine differences in pre- and postexercise lactate and hematocrit measurements. The Least Square Method was used to derive a mathematical expression for the best fit to relate VO_2 to PRL and regression analysis was applied to determine the relationship between changes in PRL concentration and MVO_2.

Results

The duration of the MVO_2 test ranged from 16 to 28 minutes. After they had completed the test, the men could be placed into seven groups according to the length of time on the treadmill (Fig. 1). Figure 1 shows the relationship between PRL concentration and duration of exercise. The exercise phase is depicted as minutes prior to exercise termination (0 time) and the recovery phase as minutes after exercise termination. The individuals in each group stopped at the same stage of the test; therefore, they shared a common duration and intensity of exercise.

Preexercise PRL levels were similar in all seven groups and remained stable for about 20 minutes of exercise. After that, levels rose acutely in all groups. In most men, the rise in PRL occurred after the exercise was terminated. In the men reaching stages 12 and 13, the concentration of PRL began to rise just prior to the end of exercise. Peak PRL levels were reached at 8 ± 3 minutes after the termination of the treadmill test. Levels remained elevated for at least

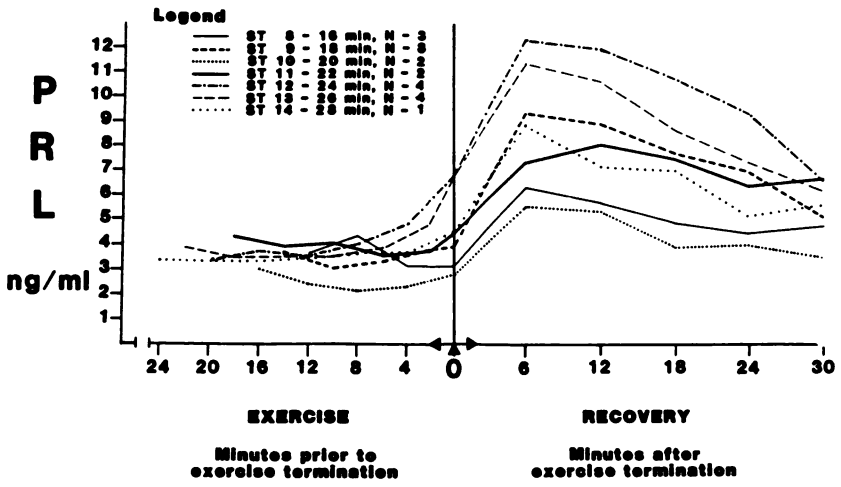

Fig. 1. Prolactin levels during the exercise and recovery phases of the treadmill test. The men in each group stopped at the same stage of the test, thereby sharing a common duration and intensity of exercise. The number of each group (ST 8-14) corresponds to the stage reached during the test. —— ST 8-16 min, N-3, -----ST 9-18 min, N-3, ·····ST 10-20 min, N-2, ——ST 11-22 min, N-2, —·—·ST 12-24 min, N-4, – – –ST 13-26 min, N-4, · · · · ST 14-28 min, N-1.

30 minutes into the recovery phase. Table 1 shows the mean rise in PRL levels for the subjects. Eighteen of the 19 subjects had a significant increase in PRL, occurring in the recovery phase of the test ($P < 0.001$).

The relationship between PRL concentration and VO_2 is shown in Fig. 2. There was a rise in PRL concentration only after the VO_2 exceeded 40 ml/kg/min. The clearest relationship between the increased PRL concentration with increasing VO_2 was seen in the subjects reaching stages 12 and 13. These subjects had the highest rate of oxygen consumption.

Using the method of Least Squares, a mathematical expression for the best fit, VO_2 and PRL concentration relationships can be obtained. The equation obtained is given by: $PRL = -0.23905 + 0.41633\ VO_2 - 0.01508\ VO_2^2 + 0.00017\ VO_2^3$. Because of the small number of men involved, this equation is best regarded as a descriptive device, not an exact relationship between PRL and VO_2. In this study, a good fit was obtained, showing that more than 90% of the change in PRL could be correlated with VO_2.

Regression analysis performed on MVO_2 and the change in PRL concentration for each of the 19 subjects showed that no significant relationship existed. A significant change occurred in lactate concentration, from a mean pre-exercise value of 1.04 ± 0.5 mmol/l to a mean post-exercise value of 7.38 ± 1.97

TABLE 1. Effect of MVO_2 Treadmill Test on Prolactin, Lactate and Hematocrit*

Exercise Phase	Prolactin (ng/ml)	Lactate (mM)	Hematocrit
Preexercise	3.7 ± 1.2	1.04 ± 0.5	43 ± 2.0
Exercise termination	4.9 ± 2.4		
Peak†	9.8 ± 5.5‡		
30-minute recovery	5.6 ± 2.6		
Postexercise		7.38 ± 1.97‡	47 ± 2.0‡

*Mean ± S.D.

†Peak values are the highest PRL levels achieved. They always occurred during the recovery phase.

‡Significantly higher than preexercise values ($P < 0.001$).

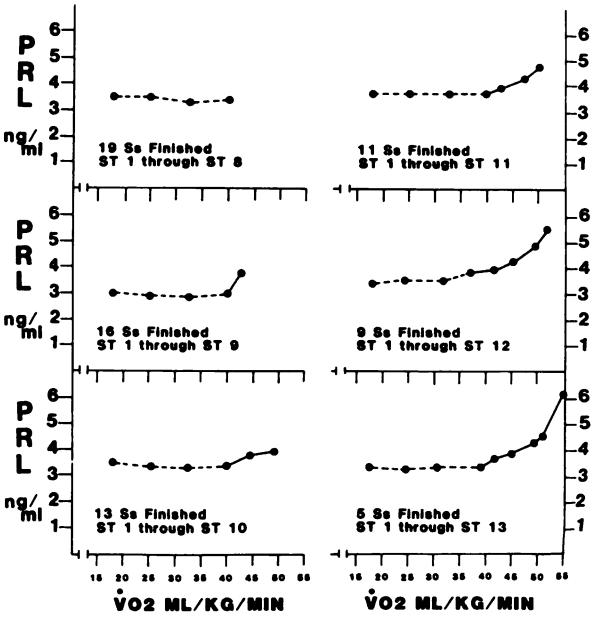

Fig. 2. The relationship between prolactin (PRL) concentration, VO_2 and stage completed during the treadmill test. All subjects (Ss) who completed the same stage of the test are grouped together.

mmol/l ($P < 0.001$). Mean hematocrit values changed significantly from 43 ± 2.0 to 47 ± 2.0 following exercise ($P < 0.001$).

Discussion

The PRL response to a standard MVO_2 treadmill stress test has been investigated. As expected, the treadmill stress test induced alterations in PRL concentration. The initial stability of PRL concentration during exercise had not been reported previously, which could be related to the fact that many studies report only pre- and postexercise levels of PRL, with no measurements carried out during exercise. The frequent measurement of PRL in the current study was responsible for revealing the more detailed pattern of PRL response to exercise.

De Meirleir et al (1985) have defined the intensity of exercise required to induce an increase in PRL levels as the anaerobic threshold—the workload at which large amounts of lactic acid are produced. They demonstrated that submaximal exercise, up to 60 minutes in duration, is not associated with an increase in systemic levels of lactic acid or PRL. This definition enables the initial stable PRL concentration seen in the current study to be explained as reflecting a subthreshold exercise intensity. De Meirleir et al (1985) further demonstrated that with graded maximal exercise to exhaustion, PRL levels rose when the anaerobic threshold was reached. These observations suggest that the duration of exercise, *per se*, is not the factor that induces the increase in PRL seen in the current study. In the protocol used for this study, the intensity of exercise reflecting a VO_2 of 40 ml/kg/min was only reached after a duration of about 20 minutes. Therefore, the identification of the two parameters, the VO_2 at the onset of PRL increase and the time of this onset, may only be identifying the more subtle parameter of anaerobic threshold.

Similarly, the study by Brisson et al in 1981 reported that the PRL response seemed to be related to work intensity in that a 50% MVO_2 workload failed to induce a PRL increase, whereas an 85% MVO_2 workload resulted in an almost doubled PRL concentration. These findings may be preliminary evidence for defining the anaerobic threshold as the work intensity necessary for a PRL increase. By definition, maximal exercise to exhaustion reaches anaerobiosis, so all the subjects in this study reached the intensity of exercise defined by De Meirleir et al (1985). The mean post-exercise lactate in this study was in excess of the 4 mmol/l criterion De Meirleir used for anaerobiosis, which lends support to the proposal that exercise intensity above the anaerobic threshold increases PRL concentration. It also demonstrates that frequent blood sampling during exercise and recovery is necessary for a refined profile of the PRL response to exercise. The pre- and immediate postexercise sampling performed in most previous studies is not adequate to pinpoint the onset of the rise in PRL during exercise or the peak concentration during recovery. Furthermore, MVO_2, considered to be a measure of physical fitness, was not related to the PRL increment in response to exercise.

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