Exercise-Induced Ovarian Dysfunction in the Rat (43029)

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Abstract. **The effect of treadmill running on estrous cycles was studied in the rat. Additional effects of cortisol acetate treatment and adrenalectomy were studied in both exercising and sedentary rats. Sedentary rats given the vehicle or cortisol acetate, or which had been adrenalectomized, all exhibited estrous cycles with diestrous phases that were uniformly less than 4 days. However exercising rats had extended estrous cycles; 50–62% of cycles were incomplete within 11 days and 78% of rats had cycles with diestrous phases that were more than 4 days long. There were no differences in duration of estrous cycles of running rats that received vehicle, received cortisol acetate, or had been adrenalectomized. We conclude that the running regimen resulted in a delay of the normal ovulatory period in rats, and that this effect of running was not affected by the presence or absence of glucocorticoids.** [P.S.E.B.M. 1990, Vol 193]

Although there are many reports that document the association of endurance training with delayed menarche, luteal dysfunction, and amenorrhea in women (1–4), there is little evidence for an association of exercise with reproductive dysfunction in animals. If such were demonstrated in laboratory animals, a model may be developed in which these relationships may be studied in more depth.

In women, duration and intensity of training (5–8), body composition and diet (9, 10), and stress associated with training (11) are factors that relate significantly to the development of amenorrhea. Serum levels of cortisol (12, 13) and endogenous opioid peptides (14–16) have been shown to be chronically elevated with increased physical activity. Androgens and prolactin are not chronically affected (1, 17, 18), but gonadotropins are generally suppressed (18) or their pattern of secretion is altered (16). Few prospective studies have been conducted, but the occurrence of anovulatory cycles in previously regularly cycling women has been demonstrated, particularly when diet was restricted (19).

There is some support for use of the rat as a model for exercise-induced anovulation. Blake *et al.* (20) found that when female rats were trained on a treadmill at 31 m/min, 5 days/week, with duration increased from 15 to 120 min over a period of 3 weeks, they did not have altered patterns of estrous cycles. Only when the rats, trained or untrained, were forced to run for up to 1 hr to fatigue at a rate of 47 m/min did they exhibit depressed luteinizing hormone levels. Whether exhausting exercise would result in suppression of ovulation is not known, but this evidence would suggest such a response. Carlberg and Fregly (21), exercising rats on a rotating wheel moving at a maximum of only 16 m/min for 1 hr daily, 5 days/week, demonstrated anovulatory periods based on vaginal smear patterns.

The present study was designed to determine the effect of running exercise on ovulation in the rat. The role of cortisol was studied to ascertain the relation between elevated levels and inhibition of ovulation in sedentary and running rats. The role of elevated hypothalamic corticotropin-releasing factor (CRF) was studied by adrenalectomizing rats at least 1 month before initiating the study. Adrenalectomy has been shown to result in increased levels of CRF in the hypothalamus (22, 23). This elevation in CRF is apparently the reason that adrenalectomized rats have a decreased LH response to ovariectomy (24). The effect of exercise on ovarian hormone secretion was evaluated by examining vaginal cytology daily. The interaction of administered cortisol as well as adrenalectomy on this response was also evaluated.

Materials and Methods

Materials. Cortisol acetate was purchased from Sigma Chemical Co., St. Louis, MO. An aqueous suspension was prepared by triturating cortisol acetate

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(CA) and adding the powder to the vehicle (100 mg/ml) while stirring the liquid with a magnetic stirrer. The suspension was stirred before drawing a measured volume into a syringe for subcutaneous injection. The vehicle was composed of 1.0% (w/v) carboxymethylcellulose and 0.9% (w/v) NaCl in water.

Animals and Experimental Treatments. Sprague-Dawley rats were obtained from Hormone Assay Lab in Chicago. They were housed in a temperature and humidity controlled environment with lights on daily between 0600 and 1800 hr. Water and rat chow (Purina) were available to the rats *ad libitum*. Adrenalectomized rats also had 0.9% NaCl in water available. Vaginal cells were obtained by lavage and examined daily for 3 weeks from all rats. Only those rats with regular estrous cycles of 4 or 5 days were selected for the study. After at least 2 complete estrous cycles had been observed, intact rats were placed randomly in treatment groups: 1—sedentary, vehicle-treated; 2—sedentary, CA-treated; 3—runners, vehicle-treated; 4—runners, CA-treated; 5—sedentary, adrenalectomized rats; and 6—runners, adrenalectomized. CA was given subcutaneously in an aqueous vehicle in a dose of 100 mg/kg daily for the last 11 days to animals designated to receive this treatment. The runners were placed on treadmills daily between 0700 and 0730 hr for 11 days and were required to run at a speed of 26 m/min for 90 min. The slope of the treadmill was 15%. Before selection for the study, rats ran for 10 min/day for 1 week; those animals that were poor runners or refused to run were eliminated from the study. The running regimen was begun for all rats on the same day regardless of the day of the estrous cycle. Adrenalectomized rats generally were not able to run for the full 90 min; they ran in 30-min segments with 10-min rest intervals. Data from adrenalectomized rats were used only if the animals had undetectable levels of serum corticosterone on the 11th day of the study. Rats were weighed daily and hormone treatment, based on body weight, was given 2 hr after the exercise bout.

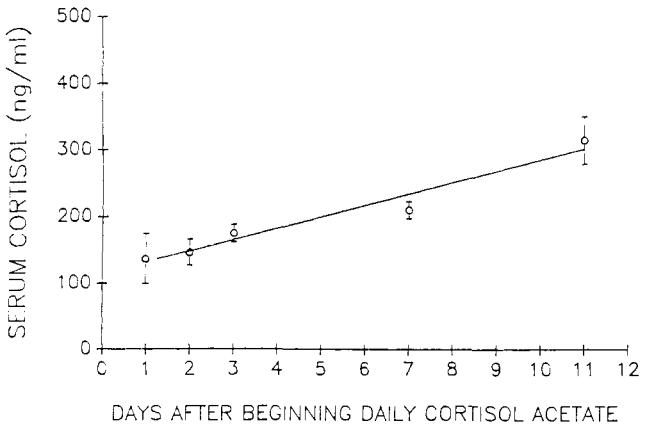

Figure 1. Concentrations of cortisol in serum after administration of cortisol acetate, 100 mg/kg body wt, daily, subcutaneously to intact sedentary rats. Blood was obtained by heart puncture from each of six rats under ketamine:xylazine (20:1) anesthesia on Days 1, 2, 3, 7, and 11 after starting CA treatment.

Vaginal smear patterns were recorded as follows: estrus, diestrus-1, diestrus-2, proestrus. In 4-day cycles 2 days of diestrus were thus recorded. In 5-day cycles 3 days of diestrous were recorded. Longer periods between typical estrus vaginal smears were characterized by a longer diestrus phase.

At the end of the 11-day treatment period, the rats were killed by decapitation 18 hr after the last exercise period, and blood and muscle tissues were obtained for analyses. Stress was minimized by frequent handling of the rats, and decapitation was performed within seconds after removal of the animals from their cages.

Other rats in Groups 1 and 2 as described above had blood drawn at frequent intervals to measure the serum levels of cortisol. These rats were anesthetized with a mixture of ketamine (100 mg/kg body wt) and xylazine (5 mg/kg body wt) and were bled by heart puncture, about 0.8 ml/bleeding.

Radioimmunoassay. Cortisol and corticosterone were assayed as described previously (25). The steroids were assayed individually by radioimmunoassay after

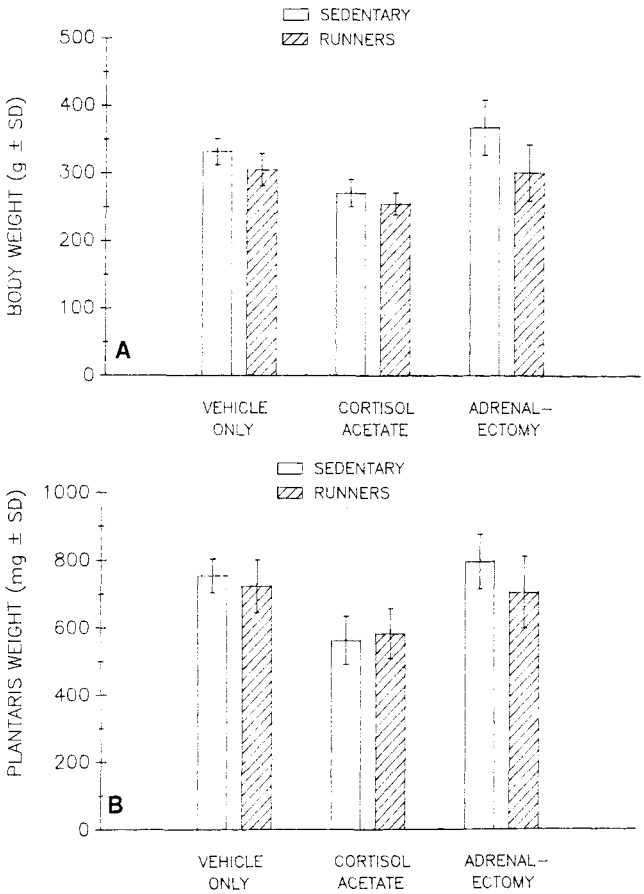

Figure 2. Body weight (A) and plantaris muscle weight (B) of sedentary and running rats at the end of the 11-day regimen. No significant differences in body weight or plantaris weight were detected between sedentary and running rats by analysis of variance. Cortisol acetate treatment significantly decreased body weight ($P < 0.01$) and plantaris weight ($P < 0.01$) in both intact sedentary and running rats.

extraction of serum and fractionation of steroids on Sephadex LH-20 according to Carr *et al.* (26).

Data Analysis. Means of animal weights and muscle mass were compared by a *t* test. Numbers of rats exhibiting a measured characteristic were compared with numbers in an appropriate control group by chi-square analysis. Differences were considered significant at the $P < 0.05$ level.

Results

Serum cortisol levels in rats in which CA was administered daily for 11 days and from which blood was drawn six times during those 11 days are shown in Figure 1. Cortisol increased rapidly from <16 ng/ml to the 150 ng/ml level and then more gradually rose to 315 ng/ml at 11 days. This treatment in both sedentary and running rats resulted in highly significant decreases in body weight and muscle mass over the 11 days compared with vehicle-treated controls (Fig. 2). It is evident that the cortisol treatment was effective in bringing about muscle wasting during the 11-day period.

Estrous cycle data for sedentary rats with and without CA treatment and for adrenalectomized rats are presented in Table I. Each rat was evaluated for two complete estrous cycles and is represented by the mean of the two cycles. Neither CA treatment nor adrenalectomy caused a significant change in the length of the estrous cycles of these rats.

The diestrous phases of 75–78% of exercising rats were 4 or more days long regardless of whether the rats received CA, the vehicle, or were adrenalectomized (Fig. 3). This response occurred in a significantly greater number of exercising rats than in sedentary rats ($P < 0.025$). Between 50 and 62% of these cycles were still incomplete after the 11-day exercise period terminated. Thus, the mean diestrus length shown in Table II was a minimum limited by the length of the period of observation.

CA administration to running rats resulted in a mean serum concentration of 246 ± 24 mg cortisol/ml on Day 11, which was similar to the serum concentration in sedentary rats after the same dosage. CA administration produced no significant effect on the length of the diestrous phases of the estrous cycles over and above that caused by running alone. Similarly, adrenalectomy did not modify the response to running.

Serum corticosterone concentrations measured at the end of the 11-day experimental period in intact, vehicle-treated rats were not different between the groups of running and sedentary rats (42 ± 14 and 53 ± 9 ng/ml, respectively). Data from adrenalectomized rats were used only if the animals had undetectable levels of serum corticosterone on the 11th day of the study.

Discussion

A model for investigation of the factors that result in an interruption of normal ovulatory function in women would benefit investigations of this phenomenon. There is circumstantial evidence for effects of glucocorticoids (12, 13), opioids (14, 15), and depleted energy reserves (9, 10) as a potential cause of ovarian dysfunction. In the present study the effect of an exercise regimen was studied for its effect on ovulation in rats over an 11-day period. An extension of the diestrous phase of the estrous cycle was the primary effect observed. In a similar study by Blake *et al.* (20) in which Wistar rats ran on treadmills for up to 2 hr/day, estrous cycles were not disrupted. This may be because of the difference in strain of rats, which has been found to be a factor with other stressors (30), or to the more gradual increase in the daily running duration in the study of Blake *et al.*.

The only treatment that extended the diestrous phase of the estrous cycles of experimental rats was exercise. Although the cortisol acetate treatment had a dramatic effect on muscle atrophy, it had no effect on estrous cycles in sedentary rats and it did not exert a synergistic effect on the extension of the diestrous phase in exercising rats. It appears that glucocorticoids do not mediate the effect of exercise on ovarian function since exercise but not cortisol acetate treatment had a clear effect within the 11-day period. Also supporting the finding of the lack of effect of glucocorticoids, in the present study the vehicle-treated running rats did not have serum corticosterone levels that were elevated over those of the sedentary rats. Previous studies have shown that cortisol or cortisol acetate could suppress gonadotropin secretion in castrate male rats (24) and monkeys (28), but the female may require more potent analogs such as dexamethasone and triamcinolone acetonide (29).

Adrenalectomy has been shown to suppress the ovariectomy-induced increase in gonadotropin secretion in the rat (24). This is presumably because adrenalectomy increases the CRF content of the hypothalamus (22, 23) which in turn inhibits gonadotropin-releasing hormone. CRF has been shown to inhibit gonadotropin secretion in rats when administered di-

Table I. Estrous Cycles of Sedentary Rats

	Intact, vehicle	Intact, CA	Adrenalectomized, vehicle
Number of animals	5	7	5
Estrous cycle length (days ± SD)	5.3 ± 0.6	5.8 ± 0.6	4.9 ± 0.4
Diestrous phase length (days ± SD)	3.3 ± 0.6	3.8 ± 0.6	2.9 ± 0.4

Table II. Estrous Cycles of Running Rats

	Intact, vehicle	Intact, CA	Adrenalectomized, vehicle
Number of animals	9	8	12
Percentage of cycles incomplete in 11 days	56	62	50
Mean diestrus length (days ± SD)	4.7 ± 2.3	5.8 ± 2.8	5.5 ± 2.5

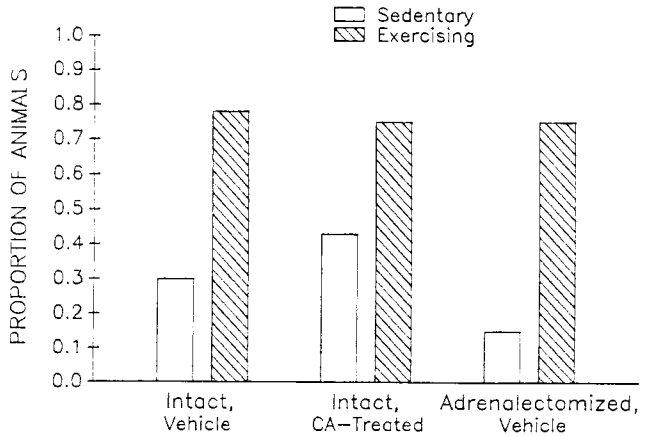

Figure 3. Proportion of rats with diestrous phases of 4 or more days.

rectly into the third ventricle of the brain (30, 31) and also when incubated *in vitro* with hypothalamic tissue (32, 33). However, adrenalectomized rats cycled normally while in a sedentary condition, indicating no interference by whatever change in gonadotropin secretion may have occurred. During exercise, adrenalectomized rats did not have a greater extension of the estrous cycles than did intact rats. Apparently, suppression of normal ovarian function as a result of exercise is not influenced by CRF, at least not with hypothalamic concentrations achieved after adrenalectomy. We conclude from these experiments that exercise is capable of suppressing ovarian function in the rat and that exercise acts through a mechanism that does not primarily involve the hypothalamic-pituitary-adrenal axis. At least in this aspect the rat may be used as a model for the study of exercise-induced anovulation.

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