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# Disruption of Estrous Cycles in Exercise-Trained Rats[1] (42058)

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*Abstract.* The female Sprague-Dawley rat was evaluated as an animal model for the menstrual irregularities that are common in women athletes. Daily vaginal smears revealed that estrous cycles were markedly disrupted in rats during a 10-week exercise training program, while cycles remained normal in sedentary rats. Compared to 9 sedentary rats, the 10 exercise-trained rats had longer mean cycle lengths and fewer estrus smears. Six of the exercise-trained rats, but none of the sedentary rats, had an "anestrus period" with more than twice the normal interval between estrus smears; one exercise-trained rat became essentially acyclic. Weight gain during the 10-week training program was lower in exercise-trained rats than in sedentary rats. Colonic temperatures, monitored at rest and during 30 min of exercise, were slightly lower in exercise-trained rats with irregular estrous cycles than in exercise-trained rats with regular cycles, indicating that unusually elevated body temperatures during exercise are not responsible for exercise-related reproductive acyclicity. It is concluded that the female Sprague–Dawley rat may be a useful animal model for the study of menstrual irregularities associated with exercise training. 

Menstrual irregularities are much more common in athletic than in nonathletic women (1). The mechanism by which athletic training affects menstrual function is not yet understood. Research in this area has thus far utilized human subjects almost exclusively. An animal model for exercise-related changes in the reproductive system would allow a variety of experiments that would not be possible with human subjects. In addition, an animal model would provide better control and intersubject homogeneity than is possible with human subjects.

We used the female Sprague–Dawley rat to assess exercise-induced changes in the reproductive cycle. Estrous cycles were monitored by daily vaginal smears in rats undergoing daily exercise training and compared with those of sedentary rats.

In women athletes, low body weight, low body fat, and weight loss are often associated with exercise-related menstrual disturbances (1, 2). Therefore, we monitored body weights in the exercising and sedentary rats.

One potential contributor to exercise-related menstrual irregularities is the elevated body temperature which occurs during exercise. Thus, we monitored colonic temperatures during exercise in the exercise-trained rats.

**Materials and Methods.** Nineteen female Sprague–Dawley rats were housed in an animal room maintained at 23 ± 1°C and illuminated from 0700 to 1900 hr. The rats were provided with standard laboratory chow and tap water *ad libitum*. Body weights were measured weekly.

Vaginal smears were examined daily throughout a 3-week pretraining period and a 10-week exercise training period. The smears were obtained between 0830 and 1200 hr using an eyedropper filled with about 1.0 ml of isotonic saline. Estrous cycle stages were defined as follows (3): diestrus stage, predominance of leukocytes; proestrus stage, predominance of nucleated epithelial cells; estrus stage, predominance of cornified cells. A smear was considered to represent the estrus stage if at least 50% of the cells appeared to be cornified. An anestrus period was defined as 9 days or more between estrus smears.

Ten rats were assigned to an exercise group and nine rats were assigned to a sedentary control group. Rats in the exercise group ran in motor-driven exercise wheels for 60 min/day, 5 days/week, for 10 weeks. The speed of the wheels was gradually increased to 16 m/min by the end of the second week; further

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increases in speed could not be maintained by the animals. The rats were observed periodically during the exercise to ensure that they continued to run. The exercise was performed between 0900 and 1130 hr. While the exercise group ran, the sedentary control rats were moved to individual cages adjacent to the exercise wheels, without food or water. Thus the control group was exposed to the same handling, isolation, and noise as the exercise group.

Temperature responses to exercise were measured in the exercise-trained rats at the end of the 10-week training period. Thermister probes were inserted about 3 cm into the colon and taped to the tail. The probes were attached to a Yellow Springs Instruments temperature recorder. Colonic temperatures were monitored immediately prior to exercise and every 5 min throughout 30 min of exercise.

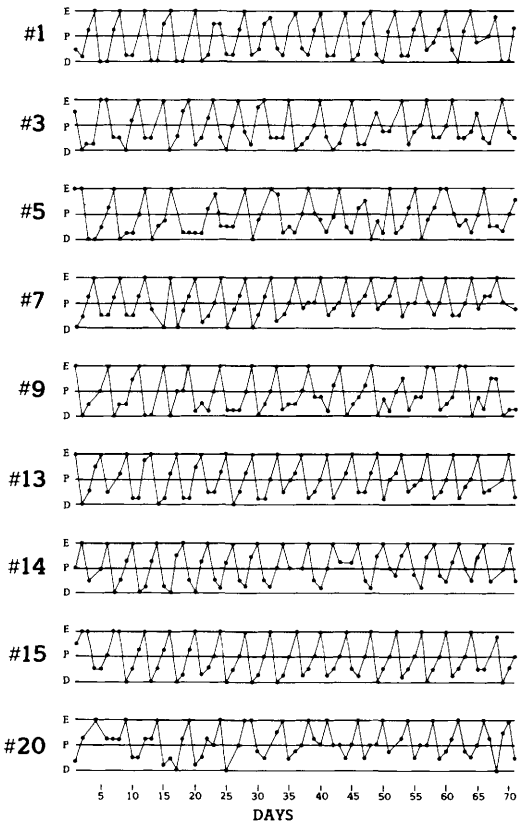


FIG. 1. Estrous cycles in the control rats during the 10-week exercise training program. E, estrus; P, proestrus; D, diestrus. Data of individual rats are shown.

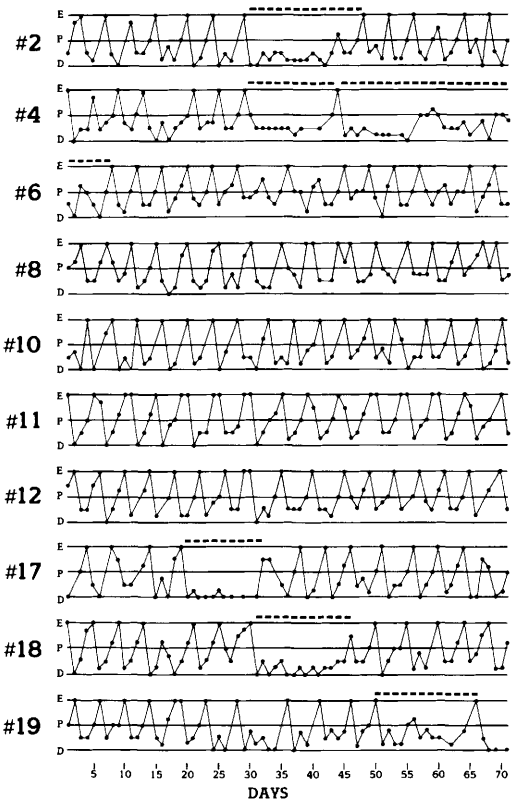


FIG. 2. Estrous cycles in the exercise-trained rats during the 10-week exercise training program. E, estrus; P, proestrus; D, diestrus. Horizontal bars denote anestrus periods. Data of individual rats are shown.

The data were analyzed with two-sample *t* tests, one-way analysis of variance, and the Newman–Keuls multiple range test (4). A significance level of 0.05 was used.

**Results.** During the 3-week pretraining period all rats had normal 4- to 5-day estrous cycles. During the 10-week experiment estrous cycles remained normal in all of the sedentary control rats (Fig. 1). However, estrous cycles were markedly disrupted in the exercise-trained rats (Fig. 2). Six exercise-trained rats exhibited at least one anestrus period during the 10-week training period. Anestrus periods ranged from 9 to 39 days in length, with a mean of 19 days. In five rats the anestrus periods were followed by a resumption of regular cycling. One rat became essentially acyclic for the remainder of the exercise training period. The anestrus periods were characterized by predominantly diestrus smears. Among the sedentary control rats

there were no cycles longer than 7 days. Cycle length during the exercise training period averaged 4.31 ± 0.11 (SEM) days in the control rats and 5.48 ± 0.56 days in the exercise-trained rats ($P < 0.05$). Number of estrus smears during the 10-week training period was 16.4 ± 0.4 for the control rats and 13.5 ± 0.9 for the exercise-trained rats ($P < 0.01$).

Body weights of the two groups of rats were similar during the pretraining period (Fig. 3). Throughout the exercise training period, mean body weights were slightly lower in the exercise-trained rats than in the control rats, and mean weights of rats with anestrus periods during the training period were slightly lower than those of exercise-trained rats maintaining regular estrous cycles. Weight gain during the 10-week training period was 38.7 ± 3.8 g (SEM) for the control group, 31.0 ± 5.0 g for the exercise-trained rats with regular estrous cycles, and 20.8 ± 3.7 g for the exercise-trained rats with anestrus periods. One-way analysis of variance and Newman–Keuls multiple range test showed that the control group and the exercise group with anestrus periods were significantly different from one another ($P < 0.05$), but neither group was different from the exercise-trained rats with regular estrous cycles.

Colonic temperatures were similar in the exercise-trained rats with regular estrous cycles and in four of the five exercise-trained rats with irregular cycles during both rest and exercise (Fig. 4). Temperatures of both groups increased by about 1.5°C during exercise. The fifth rat with irregular cycles had colonic temperatures that ranged from 1.1 to 3.5°C below the mean temperatures for the other exercise-trained rats.

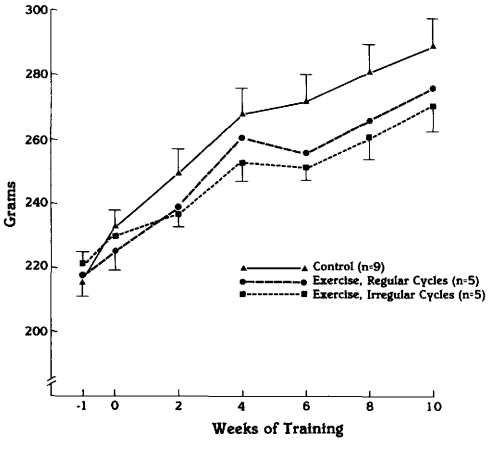


FIG. 3. Mean body weight (±1 SEM) during the 10-week exercise training program. Groups are designated in the figure.

**Discussion.** A disruption of estrous cyclicity was very definitely associated with exercise training in these rats. The disruption appeared to be somewhat similar to that which affects menstrual cycles in women athletes. A relatively large proportion of women athletes become oligomenorrheic, and a smaller number become amenorrheic for varying lengths of time (1, 5). The relatively brief anestrus periods in five rats and prolonged anestrus period in one rat in this study appear somewhat analogous to the situation in women. In amenorrheic women athletes, serum concentrations of estrogens, progresterone, and gonadotropins have most often been reported to be in the low to low-normal range (6, 7). It is likely that follicular development is minimal and ovulation seldom occurs. The pattern of vaginal smears in the exercising rats suggests a similar situation. In the rat, cornification of the vaginal epithelium results from an elevated serum estrogen concentration and is presumptive evidence of follicular maturation and ovulation (3). The absence of estrus smears and predominance of diestrus smears during the anestrus periods of the exercising rats suggest an absence of follicular development and ovulation in these animals. Thus it is possible that chronic exercise affects the hypothalamic–pituitary–ovarian axis of female rats and humans in a similar manner.

It is possible that a "psychological stress" associated with the exercise wheels was involved in the disruption of estrous cycles in the exercise-trained rats. It is well known that psychological stress can affect menstrual cycles in women (8). We minimized the difference between groups in stress exposure by moving the sedentary rats to individual cages during the exercise sessions. However, the role of stress in exercise-related reproductive acyclicity cannot be dismissed for either rats or humans.

Exercise training has previously been reported to affect the ovaries of female rats. Asahina *et al.* (9) observed ovarian atrophy,

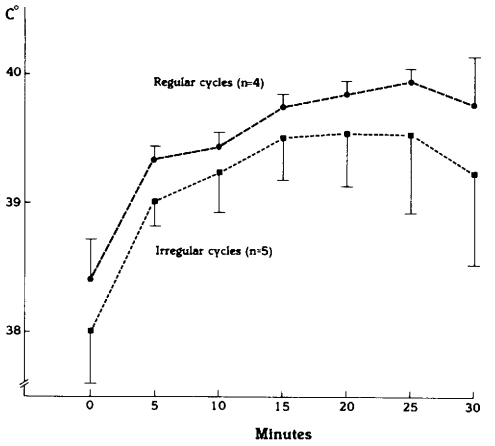


FIG. 4. Mean colonic temperatures (±1 SEM) during 30 min of exercise in the exercise-trained rats.

degeneration of ovarian follicles, and large numbers of atrophied corpora lutea in heavily exercised rats. Chandra *et al.* (10) found that ovaries taken from exercise-trained rats were reduced in weight. The ovarian tissue had lower concentrations of $\Delta^5$-3$\beta$-hydroxysteroid dehydrogenase and glucose-6-phosphate dehydrogenase, suggesting a reduction in ovarian steroidogenesis.

Low body weight, low body fat, and weight loss are often associated with exercise-related menstrual irregularities in women athletes (1, 2, 11). In this study, exercise-trained rats with anestrus periods gained less weight during the 10-week training period than did sedentary or exercise-trained rats maintaining regular estrous cycles. These data support a role for low body weight in exercise-related reproductive cycle disturbances. The interactions between body weight or body fat and reproductive cyclicity deserve further study in both humans and animals.

Colonic temperatures measured in the exercise-trained rats at rest and during exercise clearly demonstrated that unusually elevated body temperature during exercise cannot be responsible for exercise-related reproductive acyclicity. Colonic temperatures were normal in four of five exercise-trained rats with irregular estrous cycles, but considerably below normal in the fifth rat with irregular cycles. Abnormal thermoregulation has been reported in women with secondary amenorrhea associated with simple weight loss or anorexia nervosa (12). Temperature regulation in individuals with exercise-related reproductive acyclicity deserves further study, in both humans and animals.

We conclude that the female Sprague–Dawley rat may be a useful model for the study of exercise-related reproductive acyclicity. Additional studies will be required to strengthen the potential similarity between rats and humans in this regard.

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