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Exercise and Estrus Cycle Influences on the Plasma Triglycerides of Female Rats (41487)

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Abstract. Since exercise and estrogens have a significant influence on plasma triglyceride (TG) concentration, this study was performed to determine the effects of exercise on the TG titers of female rats in the four stages of the estrus cycle. Normal female rats in the various phases of the estrus cycle, ovariectomized females, ovariectomized rats receiving estradiol, and normal male rats, all of comparable age, were run to exhaustion. At the time of exhaustion, the runner and a weight-matched control were anesthetized and exsanguinated. Ovariectomized animals receiving estrogen replacement ran 61% longer than the male rats. However, this difference probably resulted from body weight differences, because when positive work was calculated, all group means were equivalent. Resting plasma TG levels were higher in normal male rats than in any other group. Ovariectomy had no effect on plasma TG levels but estrogen administration increased the concentration by 35%. Phase of the estrus cycle had no effect on resting TG levels. Exercise reduced plasma TG levels in all groups. The exercise-induced plasma TG response was not influenced by the phase of the estrus cycle. The concentration of TG at exhaustion was equivalent for all groups regardless of the preexercise TG level. These findings suggest that, during exercise, animals with high resting TG titers divert a greater portion of this fuel to oxidation than to tissue TG synthesis.

Plasma triacylglycerols (TG) supply a portion of the fuel used by tissue for energy production (1). The muscle work of exercise increases energy utilization and fuel demand. It is well documented that exercise has a lowering effect on plasma TG. One bout of exercise will significantly lower the plasma TG level in humans (2) and rats (3).

The ovarian steroid hormone estrogen has a striking, while paradoxical, influence on the level of TG in the blood. Removal of estrogenic hormones by ovariectomy or menopause is associated with an elevation in plasma TG titers (4). On the other hand, females receiving ovarian steroid hormones in the form of birth control pills have higher TG levels than females having normal menstrual cycle levels of estrogens (5). Moreover, rats receiving estrogen injections also have circulating TG titers elevated above control levels (6). Since exercise and ovarian steroid hormones have a significant influence on plasma TG levels, it was the purpose of this study to determine the influence of an exhaustive bout of treadmill running on the plasma TG levels in female rats having altered circulating levels of estrogens.

Methods. Normal female, ovariectomized female, and male Sprague-Dawley rats (Harlan–Sprague-Dawley, Madison, Wisc.) were used in this study. At the time of sacrifice all animals were 4 months of age. The mean body weight of each group is given in Table I. Since animals were age matched, it can be seen that there were mean differences in body weight with males being heavier than any other group. All animals were housed individually and provided unrestricted access to Purina rat chow and water. The animal quarters were maintained at 23 ± 1° with a 12-hr light–dark cycle (0700–1900 hr light).

To determine the influence of removal of ovarian hormones, as well as the effect of estrogen replacement in ovariectomized rats on the parameters measured, surgical ovariectomy took place when the female animals were 3 months old. This was performed by removing the ovaries through a dorsal midline incision. Ovariectomy was characterized by a very small (67.4 ± 8.5 mg) uterine weight at sacrifice when com-

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TABLE I. BODY WEIGHTS OF NORMAL FEMALES, NORMAL MALES, OVARIECTOMIZED SHAM-INJECTED (OSI) FEMALES, AND OVARIECTOMIZED ESTROGEN-INJECTED (OEI) RATS

Normal females (64)	OSI females (12)	OEI females (12)	Normal males (20)
221 ± 2.6	248† ± 4.4	216 ± 3.1	308* ± 2.7

Note. All values are mean ± SEM; number of animals per group is given in parentheses.
* Significantly heavier than all other groups ($P < 0.05$).
† Significantly heavier than normal females and OEI females ($P < 0.05$).

pared to normal control female uteri (140 ± 8.2 mg). The mean body weight of these animals was also greater than normal. The day following surgery, 13 of the ovariectomized rats began receiving daily intramuscular hindlimb injections of 5 μg of estradiol-3 benzoate (Sigma Chemical Co.) in 0.2 ml of sesame oil (OEI group). The other 12 ovariectomized animals received daily 0.2 ml sham injections of sesame oil (OSI group). The mean uterine weight of the estrogen-injected animals was 314 ± 14 mg at the time of sacrifice.

All animals were indoctrinated to the treadmill with three 10-min running bouts. Only female animals were used that had normal estrus cycles, as determined by microscopic evaluation of vaginal smears (7). This procedure was also utilized as an indirect index of the circulating ovarian hormone titers. Since the effect of exercise on the plasma TG levels in males has been reported previously (3), we included a group of age-matched males in this study as a positive control.

On the day of the exhaustive treadmill run, animals were weighed and vaginal smears were evaluated to determine their stage of the estrus cycle. Animals found to be in the same stage of the estrus cycle were paired by weight and assigned to either the runner or control group. Exercised animals were run on a Quinton 42-15 rodent treadmill at 27.34 m/min up an 8% grade until the animals could no longer avoid the shocker. When the runner was removed from the treadmill, both runner and weight-matched control were ether anesthetized. The mean time of sacrifice of each group was similar to negate the circadian rhythm effect on the data. Rats were exsanguinated by cannulation of the abdominal aorta. Blood was collected in heparinized tubes and centrifuged. Plasma was stored at −80° until subsequent analysis for triglyceride concentration. Preliminary experiments indicated that freezing had no effect on the plasma TG values. The method of Fletcher (8) was used to determine the amount of TG in each sample. The value obtained for each animal was the mean of duplicate analysis performed on two separate occasions.

All values presented are group means ± SEM. Comparison of three or more means was performed using one-way analysis of variance. If an F ratio of $P < 0.05$ was calculated, a Dunnett's post hoc test was performed (9). Two means were compared using Student's t test.

Results. The mean run times of normal female rats at each phase of the estrus cycle, OSI females, OEI females, and normal males are given in Table II. The run time of the ovariectomized rats receiving estrogen replacement was significantly greater than mean run times obtained for the male and normal females. When the phase of the estrus cycle was considered, the OEI run time was significantly greater than mean values obtained for runners in the estrus and proestrus phases of the estrus cycle.

The total amount of positive work performed for each animal was calculated from body weight, speed, and run time (up an 8% incline). The mean value for each group is found in Table III. It can be seen that no significant differences existed between group means.

Mean plasma TG concentrations for each control and exercise group are given in

TABLE II. RUN TIMES TO EXHAUSTION FOR NORMAL FEMALE RATS AT EACH PHASE OF THE ESTRUS CYCLE, OVARIECTOMIZED SHAM-INJECTED (OSI) FEMALES, OVARIECTOMIZED ESTROGEN-INJECTED (OEI) FEMALES, AND NORMAL MALES

	Run times (min)
Normal females	
Estrus (10)	125.6 ± 8.7
Metestrus (11)	144.3 ± 9.9
Diestrus (10)	135.5 ± 16.7
Proestrus (6)	98.8 ± 18.0
Composite (37) (all normal female runners)	129.5 ± 6.8
Ovariectomized females	
OSI (6)	141.5 ± 16.2
OEI (6)	177.8 ± 21.3*
Males (10)	110.3 ± 12.6

Note. All values are means ± SEM; number of animals per group given in parentheses.

* Significantly greater than the run times for all the normal males, and all the normal females in the estrus and proestrus phases of estrus cycle.

TABLE III. POSITIVE WORK[a] PERFORMED FOR NORMAL FEMALE RATS AT EACH PHASE OF THE ESTRUS CYCLE, OVARIECTOMIZED SHAM-INJECTED (OSI) FEMALES, OVARIECTOMIZED ESTROGEN-INJECTED (OEI) FEMALES, AND NORMAL MALES

	Positive work performed (kg·m)
Normal females	
Estrus (10)	60.6 ± 4.4
Metestrus (11)	69.4 ± 4.8
Diestrus (10)	68.5 ± 9.5
Proestrus (6)	46.7 ± 7.6
Composite (37)	63.1 ± 3.4
Ovariectomized females	
OSI (6)	74.9 ± 9.1
OEI (6)	85.6 ± 2.0
Males (10)	74.3 ± 9.0

Note. All values are means ± SEM; the number of animals per group given in parentheses.

[a] Positive work was calculated from the product of: body weight (kg) × speed (m/min) × run time (min) × grade (0.08).

Table IV. When group control values were compared, results indicated that the ovariectomized female rats receiving estrogen had significantly higher concentrations of plasma TG than sham-injected ovariectomized rats, as well as the overall composite TG value for all the normal female rats. Male control plasma TG levels were significantly higher than all other control groups with the exception of the ovariectomized females receiving estrogen. Exercise produced a significant reduction in the plasma TG levels of all groups except the females in the diestrus stage of the estrus cycle.

The decrease in plasma TG as a result of treadmill running was determined by calculating the difference in plasma TG levels between each control and weight-matched

TABLE IV. PLASMA TRIGLYCERIDE CONCENTRATIONS FOR CONTROL AND EXHAUSTED MALE RATS, NORMAL FEMALE RATS, OVARIECTOMIZED FEMALE RATS (OSI), AND OVARIECTOMIZED FEMALE RATS RECEIVING ESTROGEN INJECTIONS (OEI)

	Plasma triglyceride (mg%)	
	Controls	Runners
Normal females		
Estrus	81 ± 8.87 (7)†·§	49 ± 2.5 (6)*
Metestrus	71 ± 7.8 (8)†·§	47 ± 3.7 (9)*
Diestrus	93 ± 7.7 (8)§	69 ± 12.7 (8)
Proestrus	93 ± 9.7 (6)§	53 ± 7.1 (7)*
Composite (All normal females)	84 ± 4.3 (29)†·§	55 ± 4.2 (30)*
Ovariectomized females		
OSI	92 ± 10.4 (6)†·§	51 ± 6.6 (6)*
OEI	124 ± 16.7 (6)	63 ± 5.7 (6)*
Males	129 ± 6.6 (10)	64 ± 7.0 (10)*

Note. All values are means ± SEM; number of animals per group given in parentheses.

* Significantly reduced plasma TG level compared to corresponding control value ($P < 0.05$).

† Significantly different from plasma TG level of OEI controls ($P < 0.05$).

§ Significantly different from plasma TG level of male controls ($P < 0.05$).

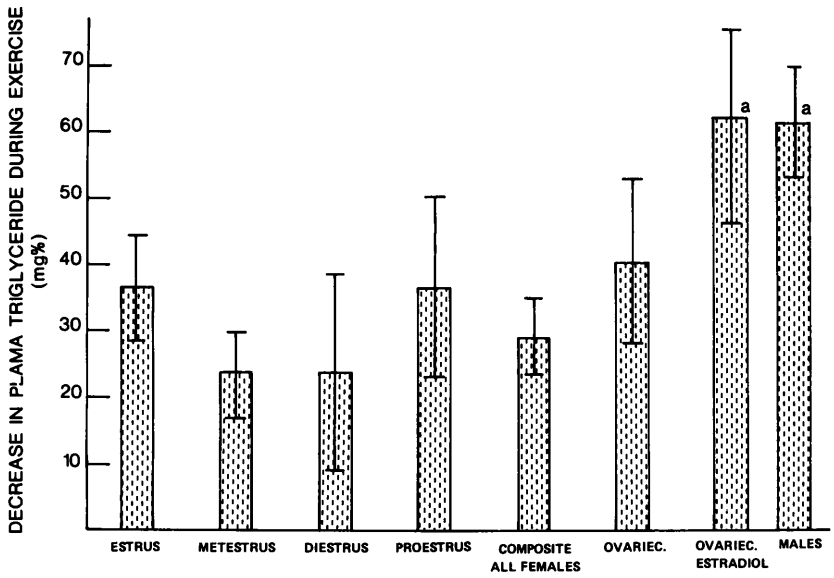

FIG. 1. The mean difference ± SEM of plasma triglyceride concentrations between runners and weight-matched controls is given for the following groups: males, ovariectomized females, ovariectomized females receiving estrogen, female rats in the various phases of the estrus cycle, and a composite value representing all normal female rats. (a) denotes a significant difference between the mean for the composite females and the group indicated.

runner. These mean values for each group are presented in Fig. 1. The TG reduction in estrogen-injected ovariectomized animals was 61.6 ± 15.5 mg%, a reduction of 49%. This exercise-induced decrease is similar to that calculated for the males of 48% (61.9 ± 8.8 mg% reduction). These decreases are significantly greater than the 20.4 mg% (35%) reduction computed for all the normal females.

Discussion. In the female rat, ovarian steroid hormones are responsible for the estrus cycle. The fluctuations that occur in estrogens and progesterone during this 4- to 5-day period are thought to be responsible for various rhythms seen in female mammalian physiology and biochemistry. Although the most pronounced influences are on uterine tissue, estrogenic rhythms have been demonstrated in body temperature (10), food intake (11), body weight (12), and voluntary running activity (13, 14). It has also been suggested that ovarian rhythms contribute to changes in blood hemoglobin content (15), plasma triglyceride titers (16), and tissue glycogen levels (17).

Since most exercise-related research avoids ovarian cycling as an experimental variable by using males or by ignoring cycling altogether, we have attempted to determine the effect of the ovarian estrus cycle on the plasma TG response to exhaustive treadmill running. The mean exercise time of ovariectomized female rats receiving estrogen was significantly greater than the means of all other groups. These findings are consistent with reports (13, 14) indicating that elevated ovarian estrogen titers increased voluntary running activity. Unfortunately, the relationship between voluntary running activity and run time to exhaustion is not known. These two parameters may not be related at all, since we found that stage of the estrus cycle had no effect on running endurance capacity. It would seem more reasonable to conclude that the increased run time in estrogen-treated rats was the result of group differences in body weight. When total positive work was estimated, taking body weight into consideration, work performance was equivalent for all groups.

It is evident from these data that the sex difference in plasma TG reported for humans (18), is also found in rats (19). The plasma TG level for male rats is approximately 50% higher than that determined in all the normal cycling females.

Estrogens have been shown to play a role in the metabolism of lipid (20, 21). However, no effect of phase of the estrus cycle on plasma TG was evident in this study. These results support the work of Punnonen (23) who found no effect of the menstrual cycle on plasma lipids. Unfortunately, Punnonen only studied three time points in the 28-day cycle. Two studies have reported that fluctuations in human TG (23) and low-density lipoproteins (24) exist during the menstrual cycle. However, differences in these parameters were not statistically significant.

While ovariectomy had no effect on plasma TG titers, daily injection of 5 μg of estradiol into ovariectomized rats significantly elevated the circulating lipid titers. Hamosh *et al.* (6) have shown that ovariectomy in rats had no effect on TG levels. This is in contrast to the works of others (25, 26), who report that prolonged removal of ovarian hormones, due to menopause or oophorectomy in humans, resulted in elevations in the concentration of plasma TG.

Ovarian hormone therapy has been used as a method of birth control. Numerous investigators, using both humans (27, 28) and experimental animals (16, 29), have reported estrogen-induced increases in plasma TG levels. We have confirmed this finding, since TG levels were elevated 37% by estrogen administration to ovariectomized rats.

The acute effect of exercise on the circulating level of TG in both man (30) and rat (3) is well documented. Recently, Terjung *et al.* (31) have shown that the uptake of plasma TG-derived fatty acids was increased in working skeletal muscle. This was supported by the results obtained in this study. Regardless of the experimental treatment, plasma TG levels were reduced as a result of one exhausting treadmill run. Phase of the estrus cycle did not quantitatively alter this exercise-induced reduction. It is interesting to note, however, that even though the initial level of TG in the plasma differed between groups, the level reached at exhaustion for all groups was approximately the same 56 mg%, a level similar to that reported by Reitman *et al.* (3) for animals that swam to exhaustion. Since all groups did approximately the same amount of work (Table III), these data (Fig. 1) suggest that significantly more energy may be derived from plasma TG in the working male and OEI female rats, than in the OSI and normal cycling females.

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