PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **174**, 53–58 (1983)

Effect of Sexual Maturation and Exhaustive Exercise on Myocardial Glycogen Metabolism (41703)

DENNIS I. GOLDBERG,* WARREN K. PALMER,‡ WILLIAM L. RUMSEY† AND ZEBULON V. KENDRICK†

Department of Physiology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104; †Biokinetics Research Laboratory, HPERD College, Temple University, Philadelphia, Pennsylvania 19122; and ‡Department of Physical Education, University of Illinois at Chicago, Chicago, Illinois 60680

Abstract. Prepubertal, pubertal, and postpubertal (28, 45, and 60 days old, respectively) rats of both sexes were run to exhaustion on a motor-driven treadmill to determine whether sexual maturation was involved in the glycogen sparing phenomenon previously reported in the myocardium of female rats receiving high doses of estrogen. Positive work was calculated and was not different in the male and female age-paired rats. Liver glycogen was significantly depleted by 92–98% in male and 96–97% in female exercising rats of all ages. The exercise bout resulted in significant depletion of glycogen in the red and white portions of the vastus lateralis muscle of male and female animals. At 45 and 60 days of age female animals had significantly more glycogen in the red muscle at exhaustion than age-paired males. Myocardial glycogen was significantly depleted by 32, 38, and 41% in prepubertal and pubertal males and prepubertal females, respectively. Pubertal females depleted myocardial glycogen by 19% ($P < 0.05$). Postpubertal male animals exhibited a 46% depletion of myocardial glycogen ($P < 0.01$), while myocardial glycogen in sexually mature females was not decreased at exhaustion. These data indicate that a myocardial glycogen sparing phenomenon exists *in vivo* in sexually mature female rats. These data further suggest that the glycogen sparing phenomenon develops in association with sexual maturation.

Glycogen sparing in skeletal muscle, cardiac muscle, and liver in response to elevated plasma free fatty acid (FFA) concentrations has been well-documented in exercised humans (1) and rats (2, 3). Net synthesis of cardiac glycogen has been reported in isolated perfused rat hearts, at high and low workloads, when fatty acid substrate was added to the perfusion medium. Cardiac glycogen concentration has been found to increase while liver and skeletal muscle glycogen decline during periods of fasting and/or starvation (4–6). Elevation of plasma FFA concentration occurs under this condition.

Lipolysis in female rat adipose tissue is increased by estrogen injection while fatty acid synthesis is decreased (7). Glycogen concentration in rat uterine muscle is elevated when estrogen is administered (8, 9). Ovariectomized female rats receiving estrogen injections demonstrated cardiac glycogen sparing when swum to exhaustion without prior feeding of lipid substrate (10). Male and sham-injected females did not demonstrate the phenomenon.

An interaction among estrogen, plasma FFA concentration, and glycogen metabolism appears to exist when pharmacological doses of the ovarian hormone are administered. Lipolysis in rat adipose tissue changes as ovarian hormone production fluctuates during the estrus cycle (7). Evidence of *in vivo* interaction between ovarian hormone production during the estrus cycle and the circadian rhythm of cardiac glycogen concentration has been reported in the rat (11). However, the effect of physiological estrogen concentrations on glycogen metabolism during exercise has not been examined. The purpose of this study was twofold: (i) to provide evidence of a sexual difference in cardiac glycogen metabolism in exercised rats not subjected to lipid prefeeding or hormonal manipulation and (ii) to examine a possible correlation between the onset of ovarian hormone production during sexual maturation and the development of cardiac glycogen sparing during exercise.

Methods. *Animal care and exercise test.* Female ($N = 46$) and male ($N = 49$) Sprague–Dawley rats (New Jersey Biological Supply or bred in our laboratory) were randomly assigned to three age groups of 28, 45, and 60 days of age (body weight 76 ± 3, 165 ± 7,

0037-9727/83 $1.50

248 ± 5 g, for females; 93 ± 3, 212 ± 6, 313 ± 8 g for males 28, 45, and 60 days old, respectively). The groups corresponded to prepubertal, pubertal, and sexually mature ages in female rats, respectively (12). The animals were raised and maintained in a 12:12 light–dark cycle (light period, 0700–1900 hr), in a controlled environment (23 ± 1°C), and fed Purina rat chow and water *ad libitum*. Each age group was randomly subdivided into a control and exercise group. Control animals remained sedentary until sacrificed. The exercise animals remained sedentary except for three 20-min periods of exposure to the motor-driven treadmill (Quinton, Seattle, WA) several days prior to their exercise bouts, in order to familiarize the animals to treadmill running.

On the day of experimentation, body weight and rectal temperature (°F) were determined (female, 100.1 ± 0.3; male, 99.9 ± 0.3), and the animals were run to exhaustion (bouts began between 0900 and 1000 hr). The exercise protocol involved a run to exhaustion on the motor-driven treadmill. The speed was initially set at 22.5 $m \cdot min^{-1}$ at an elevation of 5% for 120 min. The intensity of exercise was increased incrementally by adjusting speed and grade as indicated in Table I. The electrical shocker was used primarily at the beginning of the run, and a blast of air from the rear of the treadmill was also utilized to stimulate the rats to run. The rats were not excessively shocked at any time during the run. Exhaustion was determined by the failure of the animal to respond to either an electrical shock or a stream of compressed air. Animals were rapidly anesthetized with sodium pentobarbital (50 $mg \cdot kg^{-1}$), and rectal temperature was obtained with a mercury thermometer (Eisele, Mich.). Control animals were sacrificed between 0900 and 1000 hr, following body weight and temperature (°F) recording (female, 100.7 ± 0.5; male, 100.3 ± 0.0). Previous studies have shown that myocardial glycogen concentration is maintained during the period between sacrifice of control animals (0900–1000 hr) and exercise animals (1200–1400 hr) (11, 13).

TABLE I. PROTOCOL FOR TREADMILL RUN

Stage	Treadmill belt speed ($m \cdot min^{-1}$)	Grade (%)	Time (min)
1	22.5	5	120
2	25.0	5	60
3	25.0	10	60
4	30.0	12.5	Until exhaustion

Tissue extraction and analytical methods. Red vastus, white vastus, liver, and myocardial tissue were excised, and immediately freeze-clamped between aluminum blocks prechilled in a mixture of acetone and dry ice. Removal and freezing of the heart required less than 10 sec after opening the chest. Tissues were stored at −30°C until assayed. Glycogen was precipitated from tissue hydrolyzed in 30% KOH by cold ethanol. Glycogen content was determined by the phenol method of Lo *et al.* (14).

Statistical analyses. The results are expressed as means ± SE. An analysis of variance (3 × 2 × 2) factorial design was used to determine the influence of age, sex, and treatment upon tissue glycogen content (15). Significant differences between means ($P < 0.05$) were subjected to post hoc analysis by Tukey's HSD test. A t test for matched pairs was utilized when appropriate.

Results. Rectal temperature was significantly elevated in exhausted rats in all age groups for both sexes (Δ °F; females + 3.6; males + 3.4; $P < 0.01$). To minimize the effect of sex differences in body weight upon exercise, positive work (kilogram · meters) was calculated (kilograms body weight × meters per minute × minutes duration of exercise bout × percentage grade of treadmill elevation). The time required to produce exhaustion was longer in female rats (females, 259 ± 30, 330 ± 40, 226 ± 27 min; males, 218 ± 30, 265 ± 31, 127 ± 28 min; for 28-, 45-, and 60-day-old animals, respectively). Since exercise intensity increased with time, the females performed similar amounts of total work to that of the heavier, age-paired males (females, 36 ± 5, 102.2 ± 3, 61 ± 7 kg · m; males, 39 ± 8, 83 ± 13, 56 ± 8 kg · m; for 28-, 45-, and 60-day-old animals, respectively). In addition, the amount of work performed during the last 60 min of exercise was similar in all age-matched groups (10.5, 36.5, and 23.6 kg · m in 28-, 45-, and 60-day males; 13.8, 36.9, and 22.4 kg · m in 28-, 45-, and 60-day females, respectively).

Liver response (Figs. 1b, 2b). Glycogen concentration in the livers of animals sacrificed at rest was significantly lower in prepubertal (28 days) animals than in pubertal

or sexually mature animals of the same sex ($P < 0.05$). The exhaustive treadmill exercise run resulted in significant degradation ($P < 0.01$) of liver glycogen in all animals. Glycogen concentration was reduced by 98, 90, and 92% in 28-, 45-, and 60-day-old male animals, respectively. In 28-, 45-, and 60-day-old female animals liver glycogen content was reduced by 97, 97, and 96%, respectively. There was no effect of gender on liver glycogen concentration in either resting or exhausted animals.

Skeletal muscle response (Figs. 1a, 2a). The glycogen concentration in the red portion of the vastus lateralis muscle was similar in control animals of both sexes. Exercise induced a 72, 71, and 84% reduction in red muscle glycogen in 28-, 45-, and 60-day-old males ($P < 0.01$), respectively. Glycogen concentration decreased by 70, 54, and 60% in 28-, 45-, and 60-day-old females ($P < 0.01$), respectively. Forty-five-day-old females utilized less glycogen in the red muscle at exhaustion than comparably aged males ($P < 0.05$). Sixty-day-old females also utilized less glycogen in the red muscle than comparably aged males. The difference in glycogen utilization at exhaustion was greater in the sexually mature male and female animals ($P < 0.01$) than in the pubertal animals.

The glycogen concentration in the white portion of the vastus lateralis muscle in control females was higher than in comparably aged

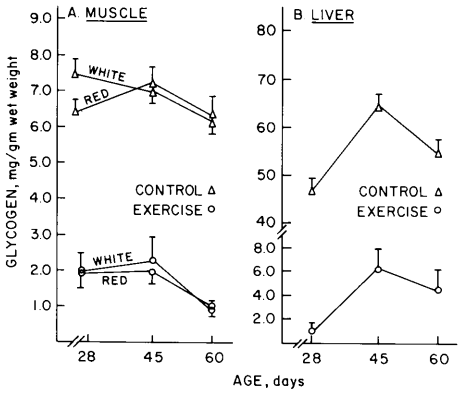

FIG. 1. Glycogen content in red and white muscle and liver from control and exercised male rats of different ages. Values are means ± SE. All exercise means are significantly different from age-paired control means ($P < 0.01$). The number of animals for each observation was between 6 and 10.

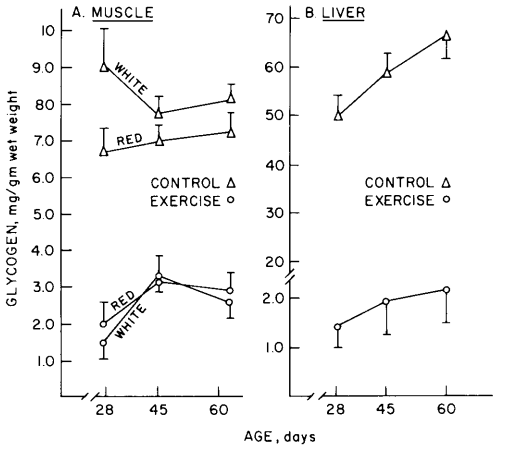

FIG. 2. Glycogen content in red and white muscle and liver from control and exercised female rats of different ages. Values are means ± SE. All exercise means are significantly different from age-paired control means ($P < 0.01$). The number of animals for each observation was between 6 and 10.

control males ($P < 0.05$). Exercise-induced degradation of white muscle glycogen was significant in all animals ($P < 0.01$). Male animals degraded 75, 68, and 90% of white muscle glycogen at 28, 45, and 60 days of age, respectively. Females hydrolyzed 83, 70, and 67% at the respective ages. Sixty-day-old females had significantly higher white muscle glycogen concentration at exhaustion than males of the same age ($P < 0.01$). However, the amount of glycogen hydrolyzed by the animals was similar in both sexes, and the difference in residual glycogen concentration probably reflected the greater initial glycogen stores in the female rats.

Myocardial glycogen response (Fig. 3). Myocardial glycogen concentration was similar in all control animals. The exercise protocol resulted in significant degradation of cardiac glycogen in males of all ages ($P < 0.01$). Myocardial glycogen was reduced 32, 38, and 46% in 28-, 45-, and 60-day-old males, respectively. The exercise protocol resulted in a 41% decrease ($P < 0.01$) in myocardial glycogen of 28-day-old female rats. Forty-five-day-old females exhibited a 19% decrease ($P < 0.05$) in myocardial glycogen. Exhaustive exercise did not induce a decrease in myocardial glycogen in sexually mature females. Sexually mature females had significantly more cardiac glycogen after exercise than mature males ($P < 0.01$).

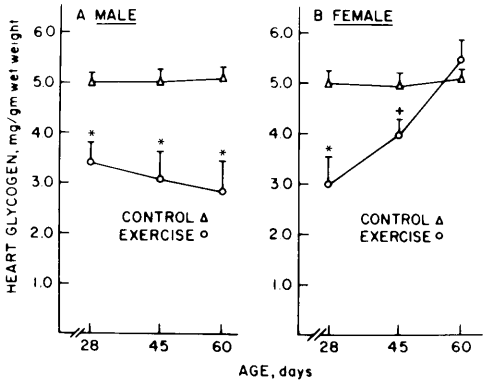

FIG. 3. Myocardial glycogen content in control and exercised male and female rats of different ages. Values are means ± SE. Asterisks indicate a significant difference ($P < 0.01$) and a cross indicates a significant different ($P < 0.05$). The number of animals for each observation was between 6 and 10.

Discussion. This study has demonstrated that exercise to exhaustion resulted in a decrease in glycogen content in liver, skeletal muscle, and heart of both sexually immature male and female, and mature male rats. Sexually mature female rats spared myocardial glycogen. There was no difference due to gender in either the amount of total work performed during exercise by age-matched animals or in the amount of work during the last 60 min of the exercise bout. The extent of glycogen degradation in liver tissue and white skeletal muscle was similar in both male and female rats of all ages. However, the response in red skeletal muscle and heart was similar only in prepubertal rats. Differences in the extent of exercise-induced degradation of red skeletal muscle glycogen became apparent as sexual maturation occurred. Sexually mature females had higher glycogen content than males in red muscle at exhaustion, even though control glycogen content was similar in both sexes.

A more distinct sex difference in glycogen content was observed in the myocardium. Myocardial glycogen concentration decreased approximately 40% in 28-, 45-, and 60-day-old males ($P < 0.01$). The response of myocardial glycogen metabolism to exercise differed in the female animals. The 28-day-old animals had not demonstrated any signs of estrus cycle activity, indicative of immature ovaries (16). Myocardial glycogen concentration decreased 41% in the prepubertal animals. This decrease was significant and similar to that observed in the males. Evidence obtained from vaginal smears indicated that the female animals in this study began estrus cycle activity between 38 and 41 days of age. The animals sacrificed at 45 days of age had been through one to two estrus cycles. The ovaries in these animals had been secreting estrogen for 4 to 7 days prior to sacrifice. Pubertal animals hydrolyzed 19% of the glycogen in their myocardial tissue. This decrease was significant, but only 50% as large as that observed in males or prepubertal females. Sixty-day-old females had been through seven or eight estrus cycles (21–32 days) at the time of sacrifice. Exercise did not cause a decrease in myocardial glycogen, indicating that the glycogen sparing phenomenon was fully developed in these animals. These data suggest that this *in vivo* glycogen sparing effect in sexually mature females was influenced, at least in part, by the production of ovarian hormones.

The glycogen sparing effect observed by Gorski *et al.* (10) followed 3 days of estradiol injections. The animals received pharmacologic doses of 50 μg estradiol/100 g body wt. In the present study the glycogen sparing effect developed *in vivo,* but required a longer period of time. Palmer *et al.* (11) have reported *in vivo* interactions between estrus cycle stage and the circadian rhythm of myocardial glycogen concentration.

The estrus cycle stage prior to sacrifice or exercise was not determined in either the 45- or 60-day-old females in this study. Plasma estrogen titer changes during the estrus cycle, reflecting altered levels of hormone production. Fluctuations in plasma titer, if present, were not reflected by variation in myocardial glycogen concentration. Variance in glycogen concentration within the 45- and 60-day females was similar to that in any of the other exercised groups. Variance within 45- and 60-day-old control females was also similar to that of other groups. The data suggests that the glycogen sparing response develops over a period of 1–4 weeks, and is not altered by changes in estrogen production within one estrus cycle.

This study has reported degradation of glycogen in the myocardium of male rats as a result of a run to exhaustion. Previous studies

have demonstrated similar patterns of glycogen degradation in the male myocardium as a result of exercise of different modes, intensities and durations (17–23). The occurrence of a glycogen sparing phenomenon in ovariectomized, estrogen-replaced, female rats has been reported after prolonged swimming (10). However, in a recent report, significant glycogen degradation was reported in sexually mature female rats (24).

The extent of glycogen degradation appears to be dependent upon the intensity of the exercise (25, 13). In the study of Gaesser and Brooks (24) the female rats worked at approximately a twofold greater intensity than the female rats in our study (180 kg·m in 4 hr as compared to 81.4 kg·m in 4 hr, respectively). However, at the lower workload of our study skeletal muscle glycogen degradation was not as complete (60–70%) as in the earlier study (93–95%). This was probably due to a greater contribution of lipid metabolism at the lower work intensity we used. Rectal temperature was elevated, but the animals were not hyperthermic. It is probable that exhaustion was caused by severe hypoglycemia secondary to liver glycogen degradation (96–97%) comparable to that reported by Gaesser and Brooks.

During the initial stages of exercise, animals of both sexes ran at the same speed and treadmill elevation. As a result, the heavier males were performing more work, and probably exercising at a higher percentage of their maximum oxygen consumption than age-matched females. This might alter the metabolic demands, causing greater utilization of carbohydrate as substrate by the males. However, as the females reached the later stages of exercise, they were running at higher speeds and elevations than the males. In the last 60 min of exercise, the females performed the same amount of work as the males. Brooks and White (27) have reported a high correlation between work and VO_2. The incremental increases in exercise intensity have adjusted for body weight differences, enabling the age-matched males and females to complete their exercise bouts at workloads which elicited equivalent oxygen consumptions. Thus, metabolic demands were probably equivalent in the age-matched animals, as indicated by comparable levels of skeletal muscle and liver glycogen degradation.

An alternative explanation for the glycogen sparing phenomenon is that glycogen degradation was equivalent in all animals during the early (10–20) minutes of exercise, and the animals were able to resynthesize glycogen during the longer exercise periods. However, the 45-day male and female animals ran the longest, but did not exhibit the same level of glycogen resynthesis as the 60-day females. It is possible, therefore, that the sexually mature females were able to utilize alternate substrate in the myocardium to a greater extent than the other animals during exercise of equivalent intensity.

Previous research reporting muscle glycogen sparing in male rats (2, 3) and humans (1) induced increased lipid metabolism via elevation of plasma substrate levels. Increased lipid metabolism prevents glycolysis through citrate inhibition of phosphofructokinase (28). Glycogen sparing induced by lipid metabolism would be most apparent in tissue with high oxidative capacity. In the present study, glycogen sparing was observed in red skeletal muscle, and to a greater extent in the myocardium. Since we did not measure tissue citrate levels, or the utilization of other substrates which could have altered glycogen metabolism (lactate, amino acids) we cannot exclude other possible mechanisms for glycogen sparing. We have previously presented evidence that glycogen sparing in female myocardium is present when phosphorylase activity is comparable to that in males (29). While we cannot preclude estrogenic effects on the utilization of other substrates, the myocardium is predominantly oxidative, with lipids the preferred substrate (30). It is possible, therefore, that the glycogen sparing mechanism which was observed in this study and by Gorski *et al.* (10) was due to an estrogen-mediated increase in lipid metabolism.

In summary, we have demonstrated that treadmill running to exhaustion at moderate intensity resulted in significant degradation of myocardial glycogen in sexually immature male and female and mature male rats while sexually mature female animals exhibited a glycogen sparing phenomenon. Since neither estrogen or tissue citrate levels were assessed in this study, it is not clear to what extent this particular hormone or other mechanisms were involved in the glycogen sparing phenomenon.

However, this phenomenon appears to be related to the process of sexual maturation.

The authors would like to thank Dr. E. O. Fuller for her advice in the preparation of this manuscript. This work was supported in part by the Biokinetics Research Laboratory Funds.

1. Costill DL, Coyle E, Dalsky G, Evans W, Fink W, Hoopes D. Effects of elevated plasma FFA and insulin on muscle glycogen usage during exercise. J Appl Physiol **43**:695–699, 1977.
2. Rennie MJ, Winder WW, Holloszy JO. A sparing effect of increased free fatty acids on muscle glycogen content in exercising rat. Biochem J **156**:647–655, 1976.
3. Hickson RC, Rennie MJ, Conlee RK, Winder WW, Holloszy JO. Effects of increased plasma fatty acids on glycogen utilization and endurance. J Appl Physiol **43**:829–833, 1977.
4. Evans G. The glycogen content of the rat heart. J Physiol (London) **82**:468–480, 1934.
5. Gold AJ, Yaffe SR. Effects of prolonged starvation on cardiac energy metabolism in the rat. J Nutr **108**:410–416, 1977.
6. Conlee RK, Tipton CM. Cardiac glycogen repletion after exercise: Influence of synthase and glucose-6-phosphate. J Appl Physiol: Resp Environ Exercise Physiol **42**:240–244, 1977.
7. Hansen FM, Fahmy N, Nielsen JH. The influence of sexual hormones on lipogenesis and lipolysis in rat fat cells. Acta Endocrinol **95**:566–570, 1980.
8. Bo WJ, Krueger WA, Garrison BM. Effect of repeated injections of estrogen on uterine glycogen. Endocrinology **59**:381–382, 1973.
9. Parvez S, Parvez H, Youdim MBH. Adrenal pituitary implication for maintenance of tissue glycogen stores in cyclic and pregnant rats. J Steroid Biochem **7**:653–658, 1976.
10. Gorski J, Stankiewicz B, Brycka R, Kiczka K. The effect of estradiol on carbohydrate utilization during prolonged exercise in rat. Acta Physiol Pol **27**:361–367, 1976.
11. Palmer WK, Goldfarb AH, Ivy JL. Circadian and ovarian influences on tissue glycogen in female rats. Endocrinology **105**:1254–1261, 1979.
12. Farris EJ. Breeding of the rat. In: Griffith J, Farris EJ, eds. The Rat in Laboratory Investigation. New York, Hafner, 2nd ed, pp1–19, 1949.
13. Segel LD, Cheung A, Mason DT, Amsterdam EA. Cardiac glycogen in Long-Evans rats: Diurnal pattern and response to exercise. Amer J Physiol **229**:398–401, 1975.
14. Lo S, Russel TC, Taylor CW. Determination of glycogen in small tissue samples. J Appl Physiol **28**:231–246, 1970.
15. Snedecor GW, Cochran WG. Statistical Methods. Ames, Iowa State Univ. Press, 6th ed, 1967.
16. Turner CD, Bagnara JT. General Endocrinology. Philadelphia, Saunders, 6th ed, pp456–468, 1976.
17. Conlee RK, Tipton CM. Influence of fasting and hormone deficiency on myocardial glycogen levels in rats. Proc Soc Exp Biol Med **149**:473–475, 1975.
18. Judd WT, Poland JL. Myocardial glycogen changes with exercise. Proc Soc Exp Biol Med **140**:955–957, 1972.
19. Poland J, Trauner D. The effects of prior exercise on myocardial glycogenesis during a fast. Proc Soc Exp Biol Med **136**:1100–1102, 1972.
20. Poland J, Trowbridge C, Poland JW. Substrate repletion in rat myocardium, liver and skeletal muscles after exercise. Canad J Physiol Pharmacol **58**:1229–1233, 1980.
21. Reitman J, Baldwin KM, Holloszy JO. Intramuscular triglyceride utilization by red, white and intermediate skeletal muscle during exhausting exercise. Proc Soc Exp Biol Med **142**:628–631, 1973.
22. Segel LD, Mason D. Effects of exercise and conditioning on rat heart glycogen and glycogen synthase. J Appl Physiol: Resp Environ Exercise Physiol **44**: 183–189, 1978.
23. Terjung RL, Baldwin KM, Winder WW, Holloszy JO. Glycogen repletion in different types of muscle and in liver after exhausting exercise. Amer J Physiol 226:1387–1391, 1974.
24. Gaesser GA, Brooks GA. Glycogen repletion following continuous and intermittent exercise to exhaustion. J Appl Physiol: Resp Environ Exercise Physiol **49**(4):722–728, 1980.
25. Poland JL, Blount PH. Glycogen depletion in rat ventricles during graded exercise. Proc Soc Exp Biol Med **121**:560–562, 1966.
26. Terjung RL, Klinkerfuss GH, Baldwin KM, Winder WW, Holloszy JO. Effect of exhausting exercise on rat heart mitochondria. Amer J Physiol **225**:300–305, 1973.
27. Brooks GA, White TW. Determination of metabolic and heart rate responses of rats to treadmill exercise. J Appl Physiol: Resp Environ Exercise Physiol **45**:1009–1015, 1978.
28. Newsholme EA. The regulation of phosphofructokinase in muscle. Cardiology **56**:22–34, 1971.
29. Rumsey WL, Goldfarb AH, Kendrick ZV. Sex-related influences on exercise-induced phosphorylase conversion and associated myocardial glycogen depletion. Med Sci Sports Exercise **13**(2):71, abstr, 1981.
30. Neely JR, Morgan HE. The relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle. Annu Rev Physiol **36**:413–459, 1974.

Received March 11, 1983. P.S.E.B.M. 1983, Vol. 174.