

Effect of Pregnancy and Exercise on Actomyosin, Nucleic Acid, and Glycogen Content of the Rat Heart¹ (35329)

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Although the effects of exercise on cardiac contractile and metabolic processes have been investigated [*e.g.* (1-3)] there is a paucity of information available concerning the effect of pregnancy on these processes. Investigations were undertaken to determine if pregnancy, or exercise superimposed on pregnancy, would have any significant effect on the actomyosin, nucleic acid, or glycogen content of the rat heart.

Methods and Materials. Female Holtzman rats (195-210 g) were fed Purina laboratory chow and water *ad libitum*. Groups consisted of rats exercised (E) 7 or 21 days, rats pregnant (P) for 7, 14, or 21 days, and pregnant rats exercised (PE) from days 0-6, 7-13, or 14-20 of pregnancy. Daily vaginal smears were taken and the presence of sperm in the vagina 8-12 hr after suspected copulation was used as the criteria for pregnancy and designated as day "0." Exercised rats were run at 15 m/min for 1000 m daily during a single exercise period on a Collins rodent treadmill, Model 297. All exercised rats were sacrificed 24 hr after the last exercise period; normal rats were included with each sacrifice.

Rats were anesthetized with sodium pentobarbital (shown not to alter cardiac glycogen levels (4)), the abdomen was opened, and an aortic blood sample was obtained. The thoracic cavity was opened quickly and the heart was excised below the atria, cut into three pieces, and gently blotted. Ventricle sections were as follows: approximately 75 mg from the apex was frozen on Dry Ice for subsequent nucleic acid determinations; approximately 100 mg were weighed and placed

in 2 ml of 30% KOH for glycogen determination; and the remaining (basal) tissue was trimmed of vessels, weighed, chilled, and minced for immediate actomyosin determination. The time from opening the chest cavity to placing the second heart slice in KOH was 20-30 sec. One placenta was taken from each of the 14-P and 21-P rats, blotted, weighed, and prepared for glycogen determination. The conceptus, fetuses, and remaining placentas were weighed separately.

Nucleic acids were extracted (5) and determined with phloroglucinol reagent (RNA) and by the Burton method (DNA) employing Pabst yeast RNA and Sigma Salmon sperm DNA as standards (6). Blood glucose, tissue glycogen, and tissue protein levels were determined by standard procedures (4). Actomyosin was extracted by Grimm and co-worker's modification (7) of the method of Benson *et al.* (8). "ATP sensitivity" of the extracted actomyosin was determined by viscosimetric analyses as an indication of the actin content. Validity of this method of actomyosin characterization has been established by several laboratories [*e.g.* (7)].

Results. Absolute adrenal gland weights of the 14-PE, 21-P, and 21-PE groups increased significantly over their respective control groups; adrenal/body weight ratios also increased significantly when 14-P and 21-P groups were exercised (Table I). All exercised (pregnant or nonpregnant) rats had significant increases in heart weight:body weight ratios over their respective nonexercised controls. Absolute heart size, however, increased significantly only when exercise was superimposed on 14-P and 21-P rats.

Hypoglycemia and elevated cardiac glycogen levels were exhibited by 14-P and 21-P rats (Table II); the values did not change with exercise except for a significant increase in cardiac glycogen in 21-P rats ($p < .01$).

¹ This work was supported by: Houston Heart Association Award no. 210-5-65-66; and Faculty Research Proposal 67-3 from the University of Houston (1967).

TABLE I. Effects of Exercise and Pregnancy on Rat Adrenal Gland, Heart, and Body Weights.

Group	No. of obs.	Increase in body wt (g)	Adrenal gland wt		Heart wt	
			(mg)	(mg/100 g of body wt)	(mg)	(mg/100 g of body wt)
C	9	16 ± 2.2 ^b	57.9 ± 2.1	25.1 ± 0.9	640.3 ± 15.8	283 ± 4.4
7-E	12	9 ± 2.1 ^a	62.0 ± 1.5	27.6 ± 1.0	656.1 ± 16.1	299 ± 3.5 ^a
C	4	45 ± 8.0	60.1 ± 2.1	23.8 ± 0.5	662.0 ± 14.7	275 ± 7.0
21-E	8	34 ± 4.4	65.9 ± 2.1	26.7 ± 0.6 ^b	676.9 ± 12.1	294 ± 4.1 ^a
C	12	20 ± 1.8	58.2 ± 1.6	26.6 ± 0.9	643.1 ± 15.5	292 ± 5.3
7-P	17	27 ± 1.7 ^b	58.1 ± 1.6	24.8 ± 0.8	657.8 ± 12.0	280 ± 5.5
C	9	16 ± 1.8	57.9 ± 1.8	26.3 ± 0.8	645.2 ± 11.3	286 ± 3.0
7-PE	12	14 ± 2.1 ^d	60.2 ± 2.1	27.0 ± 0.9	657.1 ± 14.2	299 ± 2.9 ^{bd}
C	6	27 ± 3.2	58.3 ± 2.3	26.2 ± 0.8	647.4 ± 13.8	287 ± 8.0
14-P	9	63 ± 3.0 ^e	59.1 ± 2.5	22.7 ± 1.1 ^a	658.4 ± 12.3	250 ± 3.4 ^e
C	9	30 ± 2.8	57.8 ± 3.1	25.4 ± 1.0	651.5 ± 11.9	285 ± 5.8
14-PE	11	59 ± 2.8 ^e	72.7 ± 1.6 ^{cd}	27.0 ± 0.8 ^d	718.1 ± 14.9 ^{bc}	267 ± 5.0 ^{ee}
C	6	40 ± 4.4	59.1 ± 2.3	26.1 ± 0.6	658.9 ± 32.4	282 ± 5.7
21-P	12	143 ± 6.0 ^e	65.6 ± 1.8 ^a	17.5 ± 0.7 ^e	671.6 ± 14.8	188 ± 4.1 ^e
C	8	40 ± 3.6	58.8 ± 1.4	25.9 ± 0.8	662.4 ± 16.5	281 ± 5.3
21-PE	11	131 ± 4.0 ^e	74.6 ± 4.3 ^{cf}	21.7 ± 1.2 ^{bg}	712.8 ± 15.3 ^{cf}	217 ± 8.3 ^{eg}

^b Mean ± SEM; ^a $p < .05$, ^b $p < .02$, and ^c $p < .001$ when compared with own control (C) group; ^d $p < .01$ when compared either with 7-P or 14-P groups; ^e $p < .02$ when compared with 14-P rats; ^f $p < .05$ and ^g $p < .01$ when compared with 21-P rats, respectively.

Increase in cardiac glycogen in 21-PE rats was also significantly higher than 14-P and 14-PE rats ($p < .01$, $.01$). Hypoglycemia became more prominent from 14 to 21 days of pregnancy and exercise did not augment these decreasing glucose levels.

Cardiac actomyosin (ATP sensitivity) was not altered by exercise alone or in either of the two 7-day pregnant groups (Table II). Though small fluctuations in actomyosin levels were observed in the 14- and 21-day groups, no significant or consistent pattern emerged. Total cardiac protein and actomyosin levels remained stable throughout the 21-day observation period regardless of forms of data expression.

RNA concentrations increased from 7 to 21 days of pregnancy, the increase being significant ($p < .05$) only in the 21-P rats; exercise prevented this change in the 21-PE rats (Table III). Myocardial DNA concentration decreased in the 7-E and 21-PE groups when compared with normal and 21-P controls, $p < .05$ and $< .01$, respectively.

Examination of the effects of exercise on the conceptus and its contents indicated a significant increase ($p < .01$) in total conceptus weight of 7-PE rats over 7-P rats (Table IV). Exercise had no adverse effect on the intrauterine environment as observed by the number of resorptions and/or fetal numbers and weights. Placental glycogen decreased from 14 to 21 days of pregnancy ($p < .01$) and a further decrease was seen when 21-P rats were exercised ($p < .002$).

Discussion. No consistent change was observed in blood glucose or cardiac glycogen levels during the different stages of estrus in normal rats or rats exercised for 7 days; neither did exercise affect the length of the estrous cycle. Although exercise alone did not cause a significant enlargement of the adrenal glands, the added physiological impact of exercise was evident in the 14-PE and 21-PE groups. This tissue effect probably was due to increased ACTH levels as a result of elevated plasma estrogen during pregnancy (9-11).

TABLE II. Effects of Exercise and Pregnancy on Rat Blood Glucose, Cardiac Glycogen, and Protein Levels.

Group	No. of obs.	Blood glucose (mg/100 ml)	Heart glycogen (mg/100 g of tissue)	Heart protein			
				Total protein as % wet vent.	Actomyosin as % :		ATP sensitivity
					Wet vent.	Total protein	
C	9	118 ± 2.7 ^a	493 ± 25	19.3 ± 0.7	2.56 ± 0.1	13.6 ± 0.7	82.8 ± 2.5
7-E	12	116 ± 3.9	477 ± 12	19.1 ± 0.5	2.76 ± 0.1	14.6 ± 0.6	85.3 ± 1.7
C	4	124 ± 3.3	451 ± 13	20.1 ± 0.9	2.65 ± 0.2	13.2 ± 0.5	77.3 ± 4.4
21-E	8	120 ± 4.9	447 ± 27	19.5 ± 0.6	2.70 ± 0.1	13.9 ± 0.3	79.5 ± 3.7
C	12	124 ± 3.3	470 ± 11	19.3 ± 0.6	2.82 ± 0.1	14.7 ± 0.8	81.7 ± 5.1
7-P	17	115 ± 3.0	463 ± 11	18.1 ± 0.3	2.77 ± 0.1	15.2 ± 0.4	84.2 ± 7.5
C	9	121 ± 1.9	490 ± 12	19.7 ± 0.5	2.66 ± 0.1	13.6 ± 0.4	85.7 ± 2.1
7-PE	12	121 ± 2.8	485 ± 12	19.4 ± 0.6	2.66 ± 0.1	13.7 ± 0.3	88.3 ± 1.6
C	6	117 ± 3.1	497 ± 19	18.6 ± 0.5	2.78 ± 0.1	15.0 ± 0.4	74.7 ± 2.2
14-P	9	93 ± 2.8 ^a	549 ± 14 ^a	18.6 ± 0.7	2.79 ± 0.1	15.1 ± 0.6	79.6 ± 2.2
C	9	123 ± 2.4	474 ± 21	20.4 ± 0.6	2.64 ± 0.1	12.9 ± 0.7	78.2 ± 3.1
14-PE	11	96 ± 1.9 ^c	542 ± 22 ^a	20.4 ± 0.4	2.77 ± 0.1	13.6 ± 0.6	85.1 ± 2.0
C	6	121 ± 4.9	482 ± 10	21.0 ± 0.6	2.85 ± 0.1	13.6 ± 0.8	81.4 ± 2.1
21-P	12	78 ± 1.5 ^{cd}	571 ± 25 ^b	19.8 ± 0.3	2.97 ± 0.1	15.0 ± 0.6	74.0 ± 2.8
C	8	118 ± 4.1	472 ± 8	20.1 ± 0.7	2.59 ± 0.1	13.0 ± 0.8	86.4 ± 1.7
21-PE	11	84 ± 2.8 ^{ce}	669 ± 29 ^{cef}	19.0 ± 0.6	2.66 ± 0.1	14.1 ± 0.6	82.4 ± 1.9

^a Mean ± SEM; ^a $p < .05$; ^b $p < .02$; and ^c $p < .001$ when compared with own (C) group; ^d $p < .002$ when compared with 14-P rats; ^e $p < .01$ when compared with 14-PE rats; and ^f $p < .05$ when compared with 21-P groups.

TABLE III. Effects of Fasting, Exercise, and Pregnancy on Rat Myocardial Nucleic Acids.

Group	No. of obs.	RNA ($\mu\text{g}/\text{mg}$ of wet ventricle)	DNA ($\mu\text{g}/\text{mg}$ of wet ventricle)	RNA/DNA
All control rats	16	3.18 ± 0.05 ^d	1.55 ± 0.04	2.06 ± 0.06
C	2	3.32 ± 0.18	1.43 ± 0.11 ^a	2.32 ± 0.06
7-E	4	3.16 ± 0.16	1.16 ± 0.03	2.73 ± 0.17
C	4	3.06 ± 0.11	1.45 ± 0.04	2.12 ± 0.13
F	10	3.09 ± 0.17	1.57 ± 0.06	1.98 ± 0.11
7-E + F	8	2.90 ± 0.06	1.51 ± 0.03	1.93 ± 0.03
C	4	3.23 ± 0.07	1.71 ± 0.03	1.90 ± 0.07
7-P	6	3.21 ± 0.12	1.71 ± 0.08	1.89 ± 0.07
C	2	3.19 ± 0.09	1.70 ± 0.05	1.88 ± 0.09
14-P	4	3.46 ± 0.10	1.81 ± 0.03	1.91 ± 0.11
C	2	3.21 ± 0.11	1.61 ± 0.02	2.00 ± 0.09
21-P	4	3.74 ± 0.10 ^{ab}	1.80 ± 0.06	2.08 ± 0.04
O	2	3.08 ± 0.03	1.54 ± 0.04	2.01 ± 0.04
21-PE	5	3.07 ± 0.09	1.39 ± 0.08 ^a	2.22 ± 0.08

^d Mean ± SEM; F = fasted 24 hr; ^a $p < .05$ when compared with own control (C) group; ^b $p < .01$ when compared with 21-PE group; and ^c $p < .01$ when compared with 21-P group.

TABLE IV. Effects of Exercise on the Conceptus of the Pregnant Rat.

Group	No. of obs.	Conceptus			Fetus			Placenta	
		Wt (mg or g)	% of body wt	Resorptions; av no./rat	Av no./rat	Av wt (g)	Av wt (g)	Glycogen (mg/100 g of tissue)	
7-P	17	729 ± 37 ^c	0.31 ± 0.02	—	12.4 ± 0.5	—	—	—	
7-PE	12	880 ± 32 ^a	0.41 ± 0.03 ^a	—	12.2 ± 0.4	—	—	—	
14-P	9	9.9 ± 0.8	3.8 ± 0.3	1.0 ± 0.5	10.5 ± 0.9	0.17 ± 0.02	0.20 ± 0.01	1095 ± 50	
14-PE	11	11.1 ± 0.5	4.1 ± 0.2	1.7 ± 0.4	12.5 ± 0.6	0.15 ± 0.01	0.17 ± 0.01	1071 ± 64	
21-P	12	83.6 ± 3.8	24.4 ± 0.7	0.3 ± 0.3	12.8 ± 0.8	4.7 ± 0.2	0.58 ± 0.02	455 ± 34	
21-PE	11	79.2 ± 3.3	24.1 ± 0.8	0.3 ± 0.1	11.3 ± 0.5	5.2 ± 0.1	0.60 ± 0.09	307 ± 14 ^b	

^c Mean ± SEM; ^a $p < .01$, ^b $p < .002$ when compared with respective nonexercised pregnant control group.

The fact that 14-PE rats did not exhibit greater cardiac glycogen levels than 14-P rats may be attributed to the modest hypoglycemia of pregnancy (117 vs 93 mg/100 ml). A substantial lowering of blood glucose appears necessary before an increase in cardiac glycogen occurs in exercised rats as evidenced by the low blood glucose levels vs high cardiac glycogen levels in the 21-P group. Similarly, in preliminary studies it was observed that a 24-hr fast imposed at the end of a 7-day exercise period elevated cardiac glycogen only when blood glucose levels approached those of the 21-P rats of Table II. Fasting-induced hypoglycemia increases growth hormone secretion as evidenced by depletion of the hormone from the pituitary gland in rats (12) and increased plasma levels of growth hormone in humans (13); increased cardiac glycogen levels occur simultaneously (4, 14, 15). Since plasma growth-promoting substance(s) increase during pregnancy in the rat (16), although not necessarily due to the apparent "fasted" state, one would expect a greater utilization of fatty acids (17, 18), a sparing of cardiac carbohydrates and therefore an increase in cardiac glycogen as observed in the present study (4, 14, 15, 17, 18).

The low blood glucose levels in the 21-P group as compared with the 14-P group were associated with lower placental glycogen levels in the former group and are observations similar to those reported by Hazelwood and Nelson on protein-depleted pregnant rats (19). Exercise reduced placental glycogen even further than that which can be attributed to the prolongation of pregnancy from 14 to 21 days. Thus, exercise may place an extra demand on placental glycogen depots during the last third of pregnancy for consumption by the fetuses and/or by the mother for fetal growth.

The cardiac contractile "system" was not altered significantly in any of the groups as evaluated by total protein concentration, actomyosin concentration, and ATP sensitivity, findings which are similar to those of Grimm *et al.* (2) for exercised rats. Cardiac nucleic acid and heart weight data suggest a slight enlargement by hypertrophy without hyperplasia in 7-E rats which agrees with observa-

tions of Grimm *et al.* (20) for growth of rat ventricular mass in this body weight range.

The increase in cardiac RNA levels in the 14-P and 21-P groups, the maintenance of cardiac actomyosin by estrogen in the rat (21), and the lack of change in total myocardial and/or actomyosin protein levels in the present study may indicate an increased "turnover" of protein in the heart during pregnancy. The increased heart size of 21-PE rats, therefore, would explain the unaltered RNA levels in this group.

The lowered DNA levels and the increase in heart size in 21-PE rats (above pregnant control levels) suggest a hypertrophy of the existing muscle fibers. However, in accord with data presented by Wool *et al.* (22) these alterations were not seen in those rats fasted 24 hr, a procedure known to decrease total heart weight and thereby nullify alterations of DNA levels.

It is possible that with severe physical exercise during pregnancy, fetuses may become hypoxic and suffer ontogenetic abnormalities as has been found in pregnant mice exposed to hypoxia (23). Evidently, such hypoxic conditions were not attained in this study as judged by the number of resorptions, fetal tissue weight changes, or obvious physical deformities of the fetuses.

Summary. Pregnant rats were exercised for 7 days during different gestational periods and cardiac actomyosin, nucleic acids, and glycogen were measured. Cardiac contractile protein appeared unaltered in exercised, pregnant, and pregnant-exercised groups. Myocardial RNA concentration increased during pregnancy as long as exercise did not cause cardiac hypertrophy. Rats pregnant for 14 or 21 days exhibited hypoglycemia and elevated cardiac glycogen; exercise increased cardiac glycogen and decreased placental glycogen in the last third of pregnancy. Exercising pregnant rats did not result in any obvious deleterious effects either to the mother or to the offspring.

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Received Sept. 11, 1970. P.S.E.B.M., 1971, Vol. 136.