PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 823–828 (1975)

Effects of Endurance Exercise on Serum Enzyme Activities in the Dog, Pig and Man[1] (38641)

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Since interest in exercise programs for prevention and rehabilitation of coronary artery disease is increasing, research on the interaction of exercise and coronary artery disease is becoming more important. A major need is an appropriate animal model that can be exercise-trained, instrumented for longterm cardiovascular dynamics monitoring and that is similar to man in its physiologic responses to exercise. The dog has been used in acute exercise studies (1, 2), but its capacity for exercise may be markedly greater than that of man, and the canine coronary artery distribution is considerably different from that of man (3). The rat has also been used as a model for exercise studies (4), but is not suitable for studies requiring extensive instrumentation for cardiovascular monitoring. The domestic swine has seldom been used in exercise studies but has a coronary artery distribution similar to that of man (2). We are interested in determining which of two species, the dog or the pig, would be the more appropriate animal model for studying cardiovascular adaptation during endurance exercise-training. Since the stress response to exercise as reflected by changes in serum enzyme activities is sparsely documented in the dog (1, 2) and pig, our study determined changes in serum enzyme activities after endurance exercise-training in both species and compared them with our results obtained from man. Our study sought to monitor serum enzyme activities after 5 mo of exercise-training (a) before and after exercise, daily, during a week of the exercise-training and (b) during three days of rest immediately after the selected training week.

Methods. We monitored serum enzyme activities in venous blood samples obtained from three trained mongrel dogs, three trained Duroc pigs and three well-conditioned human males before and after exercise during 5 consecutive days of running and for 3 days of rest immediately following. The adult (1-yr old) dogs, averaging 22 kg, and the 1 yr-old Duroc pigs, averaging 43 kg, were trained to run on a Quinton motor-driven treadmill (Model 18-49D) for 5 mo. The animals trained five days per week in an air conditioned room maintained at 17 ± 1°. Rectal temperature and heart rate were monitored in each animal periodically. The well-conditioned men, averaging 35-yr of age and 74 kg, normally trained from 5 to 7 days per wk, running 40–50 miles per wk over hilly terrain.

During this study the men averaged 10 miles per day at 8 mph, running over hilly terrain between 8:00 and 10:00 AM. The ambient temperature ranged between 18 and 20° with a relative humidity averaging 65%. The runners considered the runs tiring but not exhausting. The pigs and dogs ran at their normal training pace. For the pigs this was 6 miles per day at 0% grade at about 5 mph. For the dogs it was 13 miles each day at 0–8% grade at about 8 mph. All subjects exercised in a regular training program and rested 48 hr before the first day of exercise, and at the time of exercise were at least 12 hr postprandial. Heart rate and rectal temperature were recorded before and after exercise in all three species.

Venous blood samples were collected in the fasting state before and within 2–4 min after running. The blood samples were immediately centrifuged and the separated sera extracted before clotting occurred. Creatine phosphokinase (CPK), glutamic–oxaloacetic (GOT), and glutamic–pyruvic (GPT) transaminase activities were assayed spectrophotometrically at 30° (Calbiochem assay kits). Lactate dehydrogenase (LDH) and

[1] Supported in part by the National Heart and Lung Institute Program Project, Grant No. HL 12373.

TABLE I. SERUM ENZYME ACTIVITY DURING EXERCISE AND REST.

Species	Control	Preexercise[b]	Postexercise[b]	Resting
		Glutamic-oxaloacetic transaminase (mIU/mL)		
Man	11 ± 2[a]	13 ± 1	17 ± 1[c, d]	9 ± 1
Dog	13 ± 4	14 ± 2	16 ± 2	11 ± 2
Pig	14 ± 5	30 ± 15	41 ± 17	10 ± 4
		Glutamic-pyruvic transaminse (mIU/mL)		
Man	9 ± 4	12 ± 1	12 ± 1	13 ± 3
Dog	27 ± 2	29 ± 3	32 ± 2	31 ± 4
Pig	18 ± 3	22 ± 3	30 ± 5[c]	25 ± 7
		Adenylate kinase (mIU/mL)		
Man	18 ± 1	18 ± 4	17 ± 3	19 ± 2
Dog	8 ± 4	7 ± 2	10 ± 2	6 ± 0
Pig	18 ± 5	18 ± 2	44 ± 15	31 ± 6
		Lactate dehydrogenase (mIU/mL)		
Man	64 ± 1	144 ± 22[c]	104 ± 8[c]	61 ± 12
Dog	2 ± 2	29 ± 9[c]	46 ± 17[c]	2 ± 0
Pig	280 ± 84	242 ± 27	281 ± 41	245 ± 85
		Creatine phosphokinase (mIU/mL)		
Man	49 ± 2	83 ± 11[c]	120 ± 14[c, d]	52 ± 12
Dog	20 ± 5	28 ± 4	49 ± 9[c, d]	21 ± 1
Pig	187 ± 84	220 ± 63	527 ± 150	110 ± 37

[a] Values are means ± SEM.

[b] These values are means for the 5 days of exercise. Control = mean of preexercise values on the first day of exercise. Resting = mean of resting values on the third day of rest.

[c] $P < 0.05$ compared with control.

[d] $P < 0.05$ compared with preexercise mean.

adenylate kinase (AK) were assayed by the methods of Adam (5) and of Bernstein *et al.* (6), respectively. We distinguished heart and muscle LDH types with an LDH activity inhibition assay, using a modification of the rapid flow kinetic method of Bishop *et al.* (7). The organ specificity of AK was determined by measuring the inhibition of isoenzymes by anti-rabbit muscle AK serum and by *p*-hydromercuricbenzoate (6). We determined serum levels of lactate by the methods of Marbach and Weil (8). We also measured hematocrit, total hemoglobin and serum hemoglobin.

Results. The mean rectal temperatures during rest and immediately before exercise were 98.2 ± 0.1 (SEM), 101.6 ± 0.1 and 102.1 ± 0.2°F for the man, dog and pig, respectively. During each bout of running, rectal temperatures rose to peak values which averaged 102.4 ± 0.3, 106.1 ± 0.2 and 108.3 ± 0.1°F for the man, dog and pig, respectively. The rise in temperature during exercise was similar in each subject within each species and was similar on each day of the study. Preexercise (resting) heart rates for the man, dog and pig, respectively, were 62 ± 1, 98 ± 7 and 107 ± 7 beats/min. Lactate levels did not change notably from resting levels in any species immediately after exercise, ranging from 2.11 ± 0.23 to 1.57 ± 0.26 m*M*/L in man, from 2.33 ± 0.20 to 2.58 ± 0.17 m*M*/L in the dog, and 2.75 ± 0.21 to 2.32 ± 0.50 m*M*/L in the pig.

In no species did the daily levels of GOT, GPT, AK or LDH, measured before exercise daily (preexercise levels), increase successively or present a consistent pattern of change. These values are presented as mean values in Table I. Preexercise levels of CPK, however, tended to rise above the previous day's preexercise levels in the dog and man but were inconsistent in the pig (Fig. 1). The mean preexercise and post exercise levels of LDH were significantly elevated over control in man and dog, and post exercise GPT levels were above control in the pig. The mean preexercise and post exercise levels over the week of exercise were not significantly different from each other for any

enzyme in any species, except for GOT in man (Table I) and CPK levels in the dog and man. CPK rose in the dog and man from 28 ± 4 (SEM) to 49 ± 9 mIU/ml and 83 ± 11 to 120 ± 14 mIU/ml, respectively (Table I). By the third day of rest those enzymes which had not changed with exercise remained at or were not significantly different from control levels.

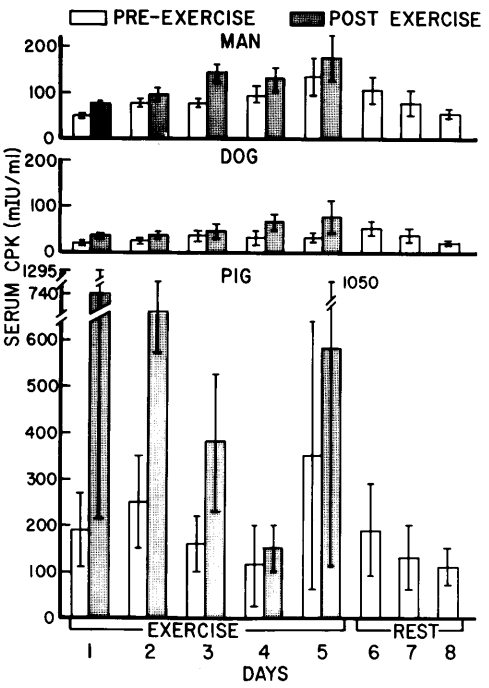

FIG. 1. Serum CPK activity in man, dog and pig. Mean values are represented by bar heights for preexercise (open bar) and post exercise (stippled bar) samples. Vertical lines = SEM. N = 3 for each bar.

Inhibition studies showed both heart and muscle types of LDH and AK in serum samples from all three species. According to Bernstein *et al.* (6) these patterns of inhibition for both AK and LDH isoenzymes indicate probable origin as the heart, liver, skeletal muscle and kidney. Changes in both types were proportionate to the change in total serum LDH activity (Table II).

The increases in hematocrit, hemoglobin and serum hemoglobin with exercise varied among the three species (Table III). In man exercise did not change hematocrit or hemoglobin, but serum hemoglobin immediately after exercise increased above preexercise levels. In the dog, hematocrit and hemoglobin after exercise were significantly above preexercise levels, but there was no significant change in serum hemoglobin. In the pig there were significant increases in all three indices after exercise.

Discussion. Schmidt and Schmidt (9) concluded that changes in serum enzyme activities after exercise depend on the amount of activity, physical condition of the subjects and the specific characteristics of the enzyme, although heat stress and hemolysis associated with exercise could also play a role (2). In our study, it is unlikely that heat stress and hemolysis were major

TABLE II. INHIBITION PATTERNS FOR ADENYLATE KINASE AND LACTATE DEHYDROGENASE AND POSSIBLE ORIGINS.

	Percentage of inhibition			
	Adenylate kinase			
	PMB[a]	AS[b]	LDH	Tissue of origin
Man				
Pre	54	66	75	Heart, Liver, Skeletal Muscle
Post	61	66	66	Heart, Liver, Skeletal Muscle
Dog				
Pre	62	32	29	Heart, Liver, Kidney, Skeletal Muscle
Post	71	38	40	Heart, Liver, Kidney, Skeletal Muscle
Pig				
Pre	48	49	55	Heart, Liver, Kidney, Skeletal Muscle
Post	32	32	43	Heart, Liver, Kidney, Skeletal Muscle

[a] PMB = *p*-Hydroxymercuricbenzoate.
[b] AS = Anti-rabbit muscle serum.

TABLE III. HEMATOCRIT, TOTAL HEMOGLOBIN AND SERUM HEMOGLOBIN.

	Hematocrit (%)		Hemoglobin (mg/100 ml)		Serum hemoglobin (mg/100 ml)	
	Pre	Post	Pre	Post	Pre	Post
Man	44.3 ± 0.7[a]	43.0 ± 0.6	14.3 ± 0.4	14.3 ± 0.3	1.19 ± 0.3	3.89 ± 0.8[b]
Dog	43.9 ± 0.9	47.5 ± 0.7[b]	15.5 ± 0.3	16.8 ± 0.4[b]	2.7 ± 0.5	4.3 ± 0.8
Pig	26.0 ± 0.9	29.5 ± 0.7[b]	8.7 ± 0.4	10.2 ± 0.2[b]	2.0 ± 0.4	6.2 ± 0.7[b]

[a] All values are means + SEM.
[b] $P < 0.05$ compared with controls.

contributing factors for the following reasons. First, rectal temperature increased with exercise similarly in each subject within each species and similarly on each day of the study, although enzyme changes varied from day to day. Second, although the mean values for serum hemoglobin were increased after exercise in man and the pig, they were not correlated with daily changes in enzyme activity. There was no significant hemolysis in the dog after exercise. Third, if hemolysis were a contributing factor we would see similar changes in AK and LDH activities, since both enzymes are found in erythrocytes. But the changes in these two enzyme activities were strikingly dissimilar, particularly in man. Hunter and Critz (10) found that training abolished the rise in LDH activity seen immediately after exercise, supporting our findings. However, Rose et al (11) reported a rise in total serum LDH activity immediately after a 26.2 mile run in a group of highly trained marathon runners. The greater severity of their exercise situation may account for the different findings. Direct evidence for these possibilities is meager; thus further studies seem appropriate. Bolter and Critz (1) and Bedrak (2) reported increases in serum LDH activity after exercise, rising to higher levels with increasing exercise stress. But their dogs had no previous exercise training, which could account for the differences we saw in our trained runners, namely, no significant changes. There are no other reports of serum LDH activity changes after exercise in trained or untrained pigs to compare with our findings.

The changes in serum AK activity we observed after exercise in the dog and pig are unique in that there are no other reports on the behavior of this enzyme in these two species. The findings in man are of interest because in normal, untrained, healthy men serum AK levels are rarely above 5 mIU/ml (6). Thus the elevated AK levels we saw may be the direct result of continued exercise training. However, the lack of change of AK activity after each exercise bout is surprising, since AK is located in the same organs as LDH and CPK. This disparity between the different enzyme activities suggests that (a) selective enzyme transit may occur across cell membranes, (b) the different enzymes may have different appearance times after stress, (c) changes in regional flow distribution may be responsible, and (d) the different enzyme activities may reflect differences in tissue concentrations. To better define the responsible factors in endurance exercise will require further study.

The fact that serum lactate levels did not increase after exercise in any subject or species indicates that the exercise elicited primarily aerobic responses. This fact and the fact that our subjects were well-conditioned must be considered when comparing our results with other studies of enzyme activity changes after exercise. For example, the decreased serum LDH we noted after exercise in man is at variance with those reported by Fowler and coworkers (12) and by Rose *et al.* (11). Fowler's study used sedentary student volunteers as subjects. The differences seen in our well-conditioned runners may be due to the following two reasons. First, greater exercise stress may be necessary before LDH will appear in the sera of trained individuals. Second, the increased cardiac output and organ blood flow that accompanies endurance exercise may cause more rapid clearance of LDH

from the serum, explaining the decrease in in LDH activity we saw after exercise.

Our results suggest that serum CPK activity is the most sensitive index of acute exercise stress in trained men and dogs. Similar changes in serum CPK activity after exercise by trained subjects had been reported in man (10) and the dog (1). In man and dog resting levels of CPK became elevated during the exercise days and then returned to control levels during the rest days. With continued physical activity CPK levels remained elevated. Similar findings in untrained individuals have been reported by Griffiths (13). In his study hospital patients confined to bed rest had CPK levels that were significantly lower than sedentary outpatients. Since CPK is a frequently used diagnostic index for pathologic conditions (14), it is particularly critical that the level of physical activity be taken into account to avoid an incorrect diagnosis. For example, during our study CPK levels in man and dog on several of the exercise days were within ranges that would be considered abnormal if the continuous training were not considered. Appropriate caution therefore, should be used in making clinical judgments from these enzyme assays in trained subjects of these species.

Our inhibition studies on AK and LDH isoenzymes after exercise in all three species confirm the findings of Bolter and Critz (1). In their study there was no change in the ratio of serum LDH isoenzymes after exercise, suggesting that the tissue sources of serum enzyme after exercise were similar to those at rest. However, Rose *et al.* (11) found that after a marathon run their human subjects exhibited an increase in skeletal muscle and liver type LDH but not heart type LDH. These differences may be related to different disappearance rates of specific LDH isoenzymes as well as to the degree of exertion.

Although significant increases in both GOT and GPT levels have been reported after exercise in man (12), we only observed similar changes in GOT in man. This difference is probably related to our subjects being exercise-trained, since other studies, using trained subjects also observed significant post exercise rises in GOT levels (10, 15) with no significant change in GPT.

Although serum enzyme changes after exercise in the dog were similar to man, the dog may not be the most appropriate model for endurance exercise studies, since its capacity for such exercise is far greater than man or the pig (16). For example, sedentary dogs can easily run 10–20 miles on a treadmill while equally sedentary men or pigs are not capable of comparable exercise. Our findings show that pigs can be endurance trained and that they exhibit responses to exercise similar to those of men. Thus, the pig may be a more appropriate model to use in future endurance exercise studies.

Summary. Daily levels of GOT, GPT, AK and LDH did not change consistently with exercise in any species. The level of CPK each day before exercise presented a consistent rise during the week of exercise in the dog and man but not in the pig. By the third day of resting, all enzyme activities were at control levels. The probable tissue of origin for AK and LDH at rest as well as after exercise was the heart, liver, skeletal muscle and kidney in all three species. Serum CPK was the most sensitive index of acute exercise stress in the trained dogs and men. We have shown that the pig, which exhibits responses to exercise resembling those of man, can be exercise trained. The pig may, therefore, be the more appropriate model for endurance exercise studies.

1. Bolter, C. P., and Critz, J. B., Proc. Soc. Exp. Biol. Med. **145**, 1359 (1974).
2. Bedrak, E., J. Appl. Physiol. **20**, 587 (1965).
3. McIntosh, H. D., Zeft, H. J., Hackel, D. B., and Kong, Y., Trans. Amer. Climatol. Ass. **79**, 124 (1967).
4. Bloor, C. M., and Papadopoulos, N. M., J. Appl. Physiol. **26**, 371 (1969).
5. Adam, H., *in* "Methods of Enzymatic Analysis" (H. U. Bergmeyer, ed.), p. 534, Academic Press, New York (1965).
6. Bernstein, L. H., Horenstein, J. M., Sybers, H. D., and Russell, P. J., J. Mol. Cell. Cardiol. **5**, 71 (1973).
7. Bishop, M. J., Everse, J., and Kaplan, N. O., Proc. Nat. Acad. Sci. U.S.A. **69**, 1761 (1972).
8. Marbach, E. P., and Weil, M. A., Clin. Chem. **13**, 314 (1967).
9. Schmidt, E., and Schmidt, F. W., *in* "Biochemis-

try of Exercise" (J. R. Poortsman, ed.), p. 216, University Park Press, Baltimore (1968).
10. Hunter, B. J., and Critz, J. B., J. Appl. Physiol. **31**, 20 (1971).
11. Rose, L. I., Bousser, J. E., and Cooper, K. H., J. Appl. Physiol. **29**, 355 (1970).
12. Fowler, W. M., Chowdhury, S. R., Pearson, C. M., Gardner, G., and Bratten, R., J. Appl. Physiol. **17**, 943 (1962).
13. Griffiths, P. D., Clin. Chim. Acta **13**, 413 (1966).
14. Tietz, N. W. (ed.), "Fundamentals of Clinical Chemistry", p. 464, W. B. Saunders, Philadelphia (1970).
15. Magazanik, A., Shapiro, Y., Meytes, D., and Meytes, I., J. Appl. Physiol. **36**, 214 (1974).
16. Dill, D. B., Pediatrics **32** (Suppl. 2), 653 (1963).

Received October 21, 1974. P.S.E.B.M. 1975, Vol. 148.