The Relationship Between Postexercise Concentration of Serum Pyruvate and Physical Fitness[1] (35845)

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It has been demonstrated that the energy requirements for muscular work are met by the oxidation of both lipids and carbohydrates (1, 2). The proportion of the energy requirement supplied by each of these substrates depends on the intensity of the exercise and on the state of physical fitness of the exercising subject (3). Fatty acids are the more important energy source in physically trained subjects; carbohydrate is more important in the unfit (3). The difference in substrate utilization is reflected by the appearance of larger amounts of the metabolites of carbohydrate metabolism (especially lactic and pyruvic acids) in the blood of untrained individuals than in the blood of trained ones after exercise at certain work levels (2, 4, 5). In most studies, trained and untrained individuals have been placed in separate groups, and conclusions have been based on results for the groups as a whole. However, it would be desirable to determine whether metabolic differences can be utilized as the basis for a biochemical test which could predict the relative state of cardiovascular fitness of individuals in a single group.

We have devised a rapid, simple technique for estimating serum pyruvate concentrations. Using this technique, we find that in response to a certain intensity of exercise on a bicycle ergometer, postexercise concentrations of pyruvate correlate significantly with each of several reliable indicators of cardiovascular fitness: maximal oxygen uptake (max $\dot{V}O_2$) (6), time to run 3 miles (7, 8), and time to run 1000 m (9). We suggest that these findings provide the basis for a useful biochemical screening test for cardiovascular fitness.

Materials and Methods. Normal male laboratory personnel and Marines were used as subjects in this study. The distribution of the subjects in the various experiments is shown in Table I. In each experiment, the subjects fell in a wide range of cardiovascular fitness. Although none of the subjects was engaged in a rigorous physical training program at the time of the study, many were taking part in regular athletic activity, and the Marines had undergone a military physical training program at some time previously. The Marines were younger and in better condition than the laboratory personnel; their preponderance in Expts. 2 and 3 accounts for the overall youth and better condition of the subjects in these experiments.

The test exercise was provided by a bicycle ergometer (Monark GCI, Monark-Crescent AB, Varberg, Sweden). The three work loads and durations of exercise are shown in Table I. Of the total number of subjects shown in Table I, 11 were tested at both 600 and 720 kilopond meters/min (kpm/min), and 16 were tested at both 720 and 900 kpm/min. No subject was tested at all three loads, and separate experiments on a given subject were at least 1 week apart.

In each experiment, the subjects were required to rest lying down for 30 to 60 min before the test exercise. Their diets were not controlled. Preexercise venous blood samples were drawn from most subjects and were obtained within 5 min of the beginning of the test exercise. The subjects then exercised at the designated load for the indicated time, after which a blood sample was drawn on each subject within 2 min after the end of

[1] The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

the exercise. (The results obtained by Keul *et al.* (2) indicate that blood pyruvate concentration does not fall below that observed at the end of exercise during the first 3 min after cessation of work.) All subjects who exercised at 600 kpm/min completed the 20-min period. Four failed to complete 30 min at 720 kpm/min, and one failed to complete 12 min at 900 kpm/min. Those who failed to complete the exercise invariably had very low max $\dot{V}_{O_2}$ and exercised for 60 to 86% of the stipulated time. Their postexercise blood samples were drawn immediately after they stopped exercising.

All blood samples were drawn from antecubital veins using minimal stasis. The blood was allowed to clot for 3 to 4 hr at room temperature. The clot was separated by centrifugation at 1000*g* for 20 min at 5 to 8°, and the serum was removed and stored at 4° until assayed within 24 hr after the experiment. Sera with visible hemolysis were rare and were discarded.

Pre- and postexercise serum pyruvate concentrations were estimated by running the undiluted serum specimens through the "blank" portion of the automated fluorometric method of Schwartz and Burns (10) for assaying serum malate dehydrogenase activity. The sampler was programmed for 20 samples/hr (2:1 sample-to-wash ratio), and a sample consisting of 0.2 *N* HCl was run after each serum specimen. We have found that this "blank" reaction utilizes lactate dehydrogenase (LDH) in the serum to convert pyruvate in the serum sample and NADH in the "blank" reagent mixture to lactate and NAD^+, respectively. The pyruvate concentration in each sample was calculated by comparing its peak with peaks derived from a series of pyruvate standards. These consisted of a range of concentrations of sodium pyruvate dissolved in albumin buffer (10) containing LDH at 1.26 IU (2 μg of protein)/ml (LDH, Type II from rabbit muscle, Sigma Chemical Co., St. Louis). These standards were run through the assay system in the same manner as were the serum specimens.

Several indices of cardiovascular fitness

TABLE I. Distribution of Subjects in the Experiments.

Expt. no.	Work load (kpm/min)	Duration of exercise (min)	No. of subjects			Age (years)		Max $\dot{V}_{O_2}$ (ml/kg/min)	
			Laboratory personnel	Marines	Total	Mean ± SD	Range	Mean ± SD	Range
1	600	20	10	5	15	31.6 ± 10.0	18–48	44.6 ± 8.2	30.5–62.9
2	720	30	16	50	66	24.4 ± 7.6	17–48	48.0 ± 6.7	30.5–62.9
3	900	12	2	14	16	22.1 ± 5.1	17–35	49.8 ± 6.0	35.5–58.7

TABLE II. Serum Pyruvate Concentrations Before and After Various Test Exercises.

Work load (kpm/min)	Time (min)	Preexercise (mg/100 ml)	Postexercise (mg/100 ml)	Sig. of difference between postexercise concentrations
600	20	0.46 ± 0.28 (15)[a]	1.08 ± 0.43 (15)	$p < 0.005$
720	30	0.70 ± 0.27 (50)	1.92 ± 0.78 (65)	
900	12	—	2.54 ± 0.55 (16)	$p < 0.001$

[a] Values are means ± SD for numbers of subjects shown in parentheses.

were determined within 1 week after the ergometer test. Each subject's max $\dot{V}_{O_2}$ was determined by the method of Taylor *et al.* (11). Additionally, most subjects ran two time trials for a 1000-m run (in gymnasium clothing) and about half ran two time trials for a 3-mile run (in military utility uniforms and boots). The better time for each distance was recorded.

Correlation coefficients relating the postexercise serum pyruvate concentrations to the various indices of cardiovascular fitness were calculated by standard methods.

Results. The results of serum pyruvate determinations at the various work loads are shown in Table II. The increase in postexercise pyruvate concentration with increasing work load is highly significant, not only for all subjects who were tested at each load (Table II) but also for those subjects who were each tested at more than one work level ($p<0.01$ for 600 vs 720 kpm/min, $N = 11$; $p<0.001$ for 720 vs 900 kpm/min, $N = 16$).

Correlation coefficients relating the postexercise pyruvate concentrations at the various work loads to the indices of cardiovascular fitness are shown in Table III for all subjects. Scatter diagrams depicting the individual data utilized to calculate these correlation coefficients are shown in Figs. 1–3. Table IV lists correlation coefficients for those subjects who were tested at more than one work load.

Discussion. The method described above for estimation of serum pyruvate concentrations was developed as a result of some observations made during previous studies (10). We are confident that this determination is measuring mainly pyruvic acid because of the high correlations which we have obtained between results of this test and those of other tests which are known to measure pyruvate (Table V). Although it has been suggested that serum not be used for pyruvate determinations (12), our results from serum (for concentrations of pyruvate before and after exercise) are similar to those reported by others for whole blood and plasma (2, 4, 13, 14). The absolute concentrations obtained using this procedure tend to be slightly different from those obtained by other methods (Table V), but this is probably due to differences in the techniques and perhaps to a small amount of interfering reaction in our procedure.

TABLE III. Correlation Coefficients Relating Postexercise Serum Pyruvate Concentrations at Designated Work Loads with Indices of Cardiovascular Fitness.

Work load (kpm/min)	Time (min)	Max $\dot{V}_{O_2}$ (ml/kg/min)	Max $\dot{V}_{O_2}$ (ml/kg of lean body wt/min)[a]	Time for run 1000 m	Time for run 3 mile
600	20	—0.306 (13)[b]	—	0.358 (11)	—
720	30	—0.644[c] (60)	—0.704[c] (51)	0.680[c] (60)	0.815[c] (28)
900	12	—0.072 (15)	—0.003 (15)	0.300 (16)	0.242 (15)

[a] Lean body weight calculated using Yuhasz's equation as stated by Wilmore and Behnke (17).

[b] Number of correlated pairs is given in parentheses.

[c] Significance of correlation coefficient, $p < 0.001$; all others not significant.

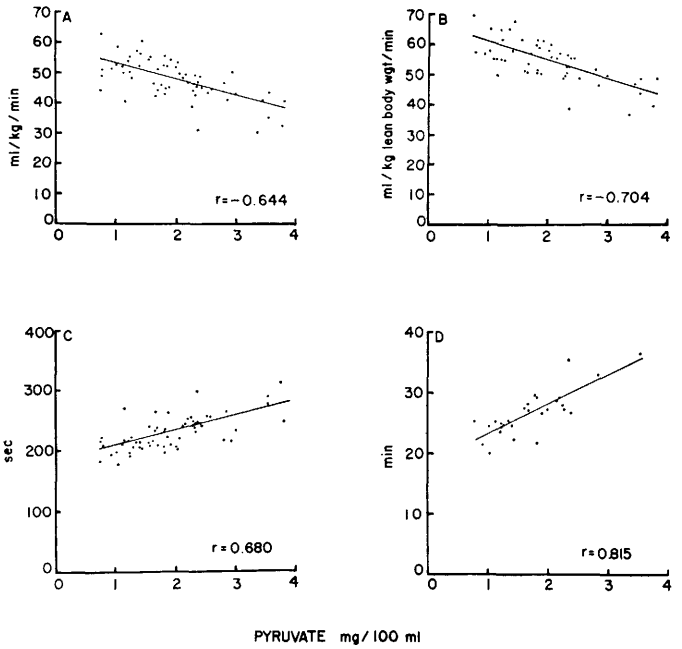

FIG. 1. Relationship between serum pyruvate concentration after exercise at 720 kpm/min (30 min) and indices of cardiovascular fitness, individual data: (A, B) max $\dot{V}O_2$; (C) time to run 1000 m; (D) time to run 3 miles.

Our automated technique makes deproteinization of specimens unnecessary, as one of the stages in the procedure is dialysis. Because LDH is supplied by the serum specimens themselves, there is no need to add exogenous LDH to the reaction system except in the standards. In fact, the addition of LDH (0.82 IU [1.3 μg of protein]/ml of albumin buffer, aspirated through the oxaloacetate line (10)) results in very little increase in peak heights for the serum samples. Sixteen random serum samples run without and with added LDH as described above gave pyruvate concentrations of 1.56 $\pm$ 0.68 and 1.79 $\pm$ 0.77 mg/100 ml (mean $\pm$ SD), respectively.

The results shown in Tables III and IV indicate that after exercise at 720 kpm/min for 30 min, serum pyruvate concentration is significantly correlated with various indices of cardiovascular fitness. Significantly lower correlation coefficients were obtained after

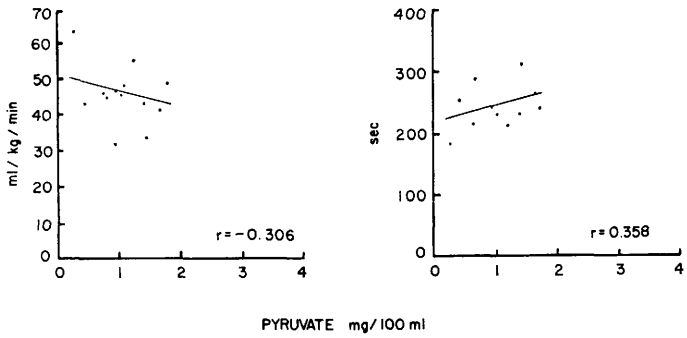

FIG. 2. Relationship between serum pyruvate concentration after exercise at 600 kpm/min (20 min) and indices of cardiovascular fitness, individual data: (A) max $\dot{V}O_2$; (B) time to run 1000 m.

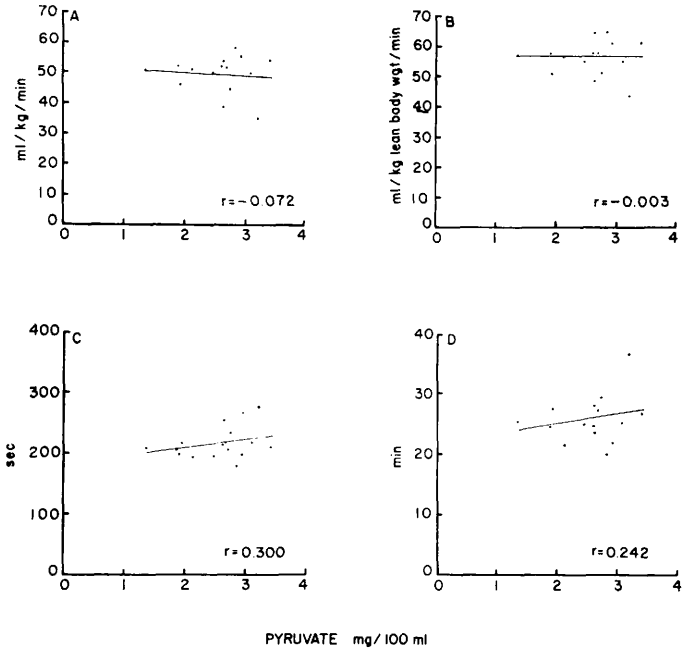

FIG. 3. Relationship between serum pyruvate concentration after exercise at 900 kpm/min (12 min) and indices of cardiovascular fitness, individual data: (A, B) max $\dot{V}O_2$; (C) time to run 1000 m; (D) time to run 3 miles.

work at the other loads, even among the relatively small number of subjects who exercised at more than one work level. These observations and the results shown in Table II agree well with the concept that different energy sources are utilized for different intensities of work in a given subject and for different states of physical fitness at a given work intensity. We postulate that at 600 kpm/min, the work load was low enough that virtually all subjects utilized substrates aerobically (mainly fatty acids) with very little anaerobic metabolism of carbohydrate. At 900 kpm/min, virtually all subjects util-

TABLE IV. Correlation Coefficients Relating Postexercise Serum Pyruvate Concentrations with Indices of Cardiovascular Fitness for Subjects Tested at More Than One Work Level.

		Subjects tested at both 600 and 720 kpm/min		Subjects tested at both 720 and 900 kpm/min			
				Max $\dot{V}O_2$		Time for run	
		Max $\dot{V}O_2$ (ml/kg/min)	Time for 1000-m run	(ml/kg/min)	(ml/kg of lean body wt/min)	1000 m	3 mile
No. of subjects tested at both loads		(11)	(9)	(15)	(15)	(16)	(15)
Work loads (kpm/min)							
600	20 min	—0.368	0.438	—	—	—	—
720	30 min	—0.722[a]	0.906[c]	—0.722[b]	—0.646[b]	0.811[c]	0.834[c]
900	12 min	—	—	—0.072	—0.003	0.300	0.242

[a] Significance of correlation coefficient: $0.01 < p < 0.05$; [b] $0.001 < p < 0.01$; [c] $p < 0.001$; unlettered coefficients not significant.

TABLE V. A Comparison of Results Obtained from Three Techniques Which Measure Serum Pyruvic Acid Concentration.

Expt. no.	No. of serum specimens	Proposed technique (mg/100 ml)	Automated GPT blank (mg/100 ml)	Pyruvate assay (mg/100 ml)	Correlation between determinations (r)
I[a]	23	1.49 ± 1.14[c]	1.37 ± 1.05		0.995
II[b]	12	1.35 ± 0.82		1.14 ± 0.67	0.992

[a] In Expt. I, 23 random serum specimens (both pre- and postexercise) were assayed for pyruvate by the proposed technique and by utilizing the blank portion of the automated fluorometric determination of glutamate-pyruvate transaminase (GPT). The GPT blank measures endogenous pyruvate (18).

[b] In Expt. II, 12 random serum specimens were assayed for pyruvate by the proposed technique and by the technique described in the booklet supplied by the Boehringer-Mannheim Corp. for pyruvate assay (13), with serum in place of whole blood.

[c] Values are means ± SD.

ized carbohydrate anaerobically, producing increased amounts of the metabolites, including pyruvate. At 720 kpm/min, subjects in better physical condition (those having high max $\dot{V}O_2$) utilized fatty acids preferentially, while those in poorer condition required anaerobic metabolism of carbohydrate, thus producing larger amounts of pyruvate than did the subjects in better condition. This was reflected in the high correlations between postexercise pyruvate concentrations in response to work at 720 kpm/min and the various indices of cardiovascular fitness.

The above hypothesis is supported by data from other sources. Williams *et al.* (15) state that anaerobic metabolism begins to occur at work levels equivalent to 40 to 45% of max $\dot{V}O_2$ in untrained subjects and at 55 to 60% of max $\dot{V}O_2$ in trained ones. From data supplied by Åstrand and Rodahl (16) we calculated that our subjects were utilizing an average of 48.1 ± 9.9% (mean ± SD) of their max $\dot{V}O_2$ at 600 kpm/min; 51.2 ± 8.2% at 720 kpm/min; and 60.3 ± 6.3% at 900 kpm/min. Clearly our intermediate load is in the best region to observe a metabolic difference between fit and unfit individuals. The fit subjects would not have reached a load requiring anaerobic metabolism, while the unfit would be past the critical load. Furthermore, at this or any other given work load, the subjects in better condition would be working at a lower percentage of their max $\dot{V}O_2$ than would the subjects in poorer condition. This fact alone could account for differences in pyruvate concentrations after exercise at a critical work load even if subjects in all states of training were to begin to utilize anaerobic metabolism at the same percentage of max $\dot{V}O_2$.

The results presented by Keul *et al.* (2) support a conclusion that our intermediate load is in the region where there is the greatest difference between trained and untrained subjects in terms of their utilization of anaerobic metabolism and appearance of pyruvate in the blood.

We found that in our conditions the actual postexercise concentrations of pyruvate were more useful than were the changes in pyruvate concentration in response to exercise. The observed preexercise concentrations of pyruvate tended to be low and somewhat variable. Friedemann *et al.* (14) have shown that food intake 1 to 3 hr before blood sampling can raise the observed pyruvate concentrations significantly. Our experiments usually commenced within 2 hr after breakfast. Because of these considerations and the fact that the correlation coefficients were always somewhat higher when postexercise pyruvate concentrations were used rather than changes in concentration, we did not draw preexercise blood samples on all subjects. This explains the absence of preexercise values at 900 kpm/min and the lower number of preexercise samples than of postexercise ones at 720 kpm/min in Table II.

Summary. Groups of male subjects in varying states of general physical condition

were exercised on a bicycle ergometer at three work loads. Pre- and postexercise concentrations of serum pyruvate were estimated by a simple, rapid automated technique which utilizes LDH in the serum specimens. Postexercise concentrations of pyruvate increased with increasing work load. After a test exercise of 720 kpm/min for 30 min, serum pyruvate concentrations correlated significantly with several indices of general physical condition: maximal oxygen uptake, time to run 3 miles, and time to run 1000 m. The techniques described may provide the basis for a simple biochemical test of cardiovascular fitness.

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