Effect of Exercise on the Serum Myoglobin, Uric Acid, and Creatine Kinase Levels in Trained Runners and Non-Runners

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This study determined the effects of different exercise levels on serum myoglobin, uric acid and creatine kinase after severe or moderate exercise. Fifteen highly trained long distance runners with maximum oxygen uptake (max VO2) 50 mL/Kg-min or over, and 15 non-runners with max VO2 of 45 mL/Kg-min or below (both groups aged 17-29 years and were all males) were selected for experimental and control groups, respectively. Both groups were subjected to severe (70–65% VO2) or moderate (50–55% VO2) exercise on a standard treadmill for 30 minutes.

Significant elevations of serum myoglobin levels were observed in both experimental and control groups that went through severe exercise, but, not in those went through moderate exercise. Higher extent of elevation was observed with the experimental group. Elevation of myoglobin in both group peaked at one hour after the exercise and returned to original levels after 24 hours.

Similar to myoglobin, significant increases of uric acid levels were observed immediately following the severe exercise in both experimental and control groups that went through severe exercise, but not in moderate exercise. The elevation reached a plateaus at 30 minutes later, then returned to original levels in 24 hours. Unlike the myoglobin, elevation of uric acid was higher in the control group than in the experimental group.

There was a slight, but not significant, increase of serum creatine kinase levels in both groups that underwent severe exercise. Also, no significant differences in the increase of serum creatine kinase level was found between the severe and moderate exercise of each group.

We had tried to find the correlation of the changes between these three elements. There was a positive correlation between the myoglobin and uric acid in severe exercise, and, between myoglobin and oreatine kinase in moderate exercise, contrary.

Key Words: Myoglobin, Uric Acid, Creatine Kinase, Exercise

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Following severe exercise, some runners may experience a stiff, painful and slow functioning leg muscle, and, even crush syndrome. It is established that crush syndrome aroused from the rise of blood or urine myoglobin level (1); however, the detail mechanism of such effect is still not understood. The effect of exercise on the myoglobin level warrants further study. If the rise of serum myoglobin level is a mechanism of overburdened muscular or heart capacity, then the various types and extent of exercise will inevitably cause the rise of myoglobin level to various degree. However, such documentations are not currently available.

Green and Fraser (2) reported the rise of serum uric acid following severe exercise to the extent of 120% maximum oxygen uptake, but little or no such rise was observed in runners who exercised with 65% of maximum oxygen uptake. It is apparent that the rise of serum uric acid is related to the extent and intensity of the exercise. But the details of such relationship require further investigation. This warrants the investigation of the relationships of serum uric acid level between the trained runner and the non-runner.

Olerud et al (3) reported the incidence of acute exertional rhabdomyolysis among recruits using several serum biochemicals as indicators. They found substantial rises of serum myoglobin, creatine kinase and two other enzymes throughout the study population following their six days of regular scheduled training. Elevation of serum muscle enzymes such as creatine kinase in strenuous exercise is felt to be due to a leakage through damaged cell membranes, as a result of decrease of adenosine-5'-triphosphate (ATP) previously dedicated to cell membrane integrity. Several investigators (4–6) reported the increases in the size and the number of ATP supplying mitochondria in skeletal muscle, thereby rendering the cell membranes more stable and preventing the efflux of enzymes during strenuous exercise. In the study of the effect of conditioning on serum creatine kinase level, Maxwell and Bloor (7) reported an increase of 98–294% above preconditioning control values of serum creatine kinase after 14 miles test run while an increase of 52–405% of serum myoglobin levels. They suggested such quantitative indices are useful in determining the conditions necessary to prevent significant rhabdomyolysis after severe exercise. Hansen and coworkers (1) reported the rise of serum enzymes such as creatine kinase, in parallel with the rise of serum myoglobin, following long distance running. However, non-runners were found to have higher rises in both serum myoglobin and creatine kinase than the trained runners. The abnormal findings are only explainable, as they (1) reported, on the basis of leakage of proteins from muscle cells to the circulation in otherwise healthy, well trained persons. It is felt that the relationships of the myoglobin, uric acid and creatine kinase with exercise need to be investigated under standardized conditions.

The purpose of the present investigation were, first, to examine the degree of changes in serum myoglobin, uric acid and creatine kinase in trained runners and non-runners under strict standardized exercise conditions; secondly, to examine the effect of the extent of exercise on such indices; thirdly, to examine the changes of such biochemical indices during the course of exercise; and fourthly, to examine the variables among serum myoglobin, uric acid and creatine kinase.

MATERIALS AND METHODS

Subjects

For initial screening, physical examinations were performed on all prospective subjects by the physicians of Taipei Municipal Ho-Ping Hospital. Those with disease and/or conditions unfit for the severe exercise were removed. Further pulmonary function tests e.g. maximum oxygen uptake were given to the remaining subjects. All of those subjects passed the tests, fifteen highly trained long distance runners with maximum oxygen uptake (max VO2) 50 mL/Kg-min or over, and 15 non-runners with max VO2 of 45 mL/Kg-min or below (both groups aged 17–29 years) were selected for experimental and control groups, respectively, of the study. For this study the volunteer subjects were all males. The body weight was 63.54±6.613 Kg in experimental group and 62.707±9.247 Kg in control group, while the body height was 173.5±6.554 cm and 171.39±5.490 cm respectively.

Methods

All selected volunteer subjects were first interviewed by trained personnel at the Taipei Municipal Ho-Ping Hospital to gather the information regarding each volunteer's personal background, family history, personal life-style, educational background, personal habits such as alcohol drinking, cigarette smoking, religious background etc. For the physical test, the treadmill during and the intensity of the exercise were all standardized. For any repeat test, at least 48 hours of waiting was required to avoid any possible bias and/or complication of the experiment. All subjects were advised not to participate in any extensive physical activities within 48 hours before the start of the study.

The measurement of maximum oxygen uptake (max VO2) and the running tests were performed at the Biomechanics Laboratory of the Research Institute of Physical Education, National Normal University, Taipei.

Test Runs

For the first test run, maximum oxygen uptake (max VO2) was used as the loading mark for the running test which was performed according to Bruce protocol. All the volunteer were advised to eat nothing since 10 o'clock of the night before until the exercise and the blood collection. Exercise was begun at a speed for 2.0 miles per hour and increased every 3 minutes, until the final stage of 6.5 miles per hour for the control group and 8.0 miles per hour for the experimental group was reached. The exhaled air was collected at each stage and submitted for CO2 and O2 analyses. For second test run, 50–55% of max VO2 was used as the loading mark for the running test, and the test was carried for 30 minutes. For the third test run, 70–75% of max VO2 was used as the loading mark and it was also carried out for 30 minutes.

Biochemical Analyses

A myoglobin radioimmunoassay kit from Biomerica (Newport Beach, CA, USA 9226) was used for the measurement of serum myoglobin level. The principle of the test is based on the immunological reaction of the antigen, 125I labeled myoglobin, which competes with the myoglobin of the sample for the specific binding sites on the antibody. The bound antigen, after equilibration, is then precipitated with a second antibody. The I-125 labeled myoglobin in the resulting precipitates is inversely proportional to the amount of unlabeled myoglobin in the standards and samples. Thus, the concentration of myoglobin in a sample is determined by comparison with a standard curve prepared by reacting varying known amounts of myoglobin with constant amounts of specific antibody.

Serum uric acid was determined by the uricase-catalyzed oxidation of uric acid to allantoin and hydrogen peroxide and was measured on a Union Carbide Centrifichemm

Table 1. Effects of Exercise on Serum Myoglobin Levels (ng/ml)

	Before	Time After Exercise (hours)					
	Exercise	Immediate	0.5	1.0	1.5	2.0	24
Experimental Group							
Moderate	41.40	50.61	43.85	46.64	45.06	46.94	44.05
exercise(SD)	±19.45	±32.13	±15.82	±16.26 #	±18.51 #	±16.84 #	±15.50 #
Severe	42.10	62.13 *	59.24 *	69.64 *	64.23 *	64.64 *	52.61 *
exercise(SD)	±12.60	±31.39	±32.58	±35.18 #	±31.71 #	±33.17 #	±17.83 #
Control Group							
Moderate	39.01	40.50	37.82	43.49	39.52	40.85	35.86
exercise(SD)	±25.84	±11.25	±18.36	±18.74 #	±15.63	±12.06 #	±15.79
Severe	35.74	42.39	47.16 *	58.73 *	58.66 *	61.12 *	33.18
exercise	±10.37	±18.48	±17.67	±23.94 #	±34.74	±28.79 #	±11.38

1. *: $p < 0.05$ in a comparison of value at each interval with start value.
2. # : $p < 0.05$ in a comparison at each interval between different exercise.

Table 2. Effects of Exercise on Serum Uric Acid Levels (mg/dL)

	Before	Time After Exercise (hours)					
	Exercise	Immediate	0.5	1.0	1.5	2.0	24
Experimental Group							
Moderate	5.67	5.77	5.73	5.76	5.73	5.57	5.91
exercise(SD)	±1.38	±1.40 #	±1.46 #	±1.36 #	±1.46 #	±1.35	±1.35
Severe	5.75	6.37	6.54	6.55 *	6.47	5.97	5.76
exercise(SD)	±1.62	±1.70 #	±1.71 #	±1.70 #	±1.69 #	±1.93	±1.48
Control Group							
Moderate	5.83	6.13	6.33	5.95	5.87	5.89	5.77
exercise(SD)	±1.24	±1.25	±1.23 #	±1.63 #	±1.62 #	±1.53 #	±1.23
Severe	6.01	6.56	7.31 *	7.34 *	7.24 *	7.16 *	6.27
exercise(SD)	±1.45	±1.06	±1.21 #	±1.27 #	±1.38 #	±1.25 #	±1.40

1. *: $p < 0.05$ in a comparison of value at each interval with start value.
2. # : $p < 0.05$ in a comparison at each interval between different exercise.

Table 3. Effects of Exercise on Serum Creatine Kinase Levels (IU/L)

	Before	Time After Exercise (hours)					
	Exercise	Immediate	0.5	1.0	1.5	2.0	24
Experimental Group							
Moderate	241.8	270.3	245.6	253.4	253.6	251.9	233.5
exercise(SD)	±124.6	±143.4	±131.7	±123.7	±130.0	±128.4	±140.6
Severe	256.8	291.4	261.7	274.1	278.0	269.9	276.0
exercise(SD)	±180.0	±212.0	±183.6	±203.6	±206.8	±196.1	±229.9
Control Group							
Moderate	196.0	206.9	188.5	194.9	199.0	198.9	164.5
exercise(SD)	±104.5	±109.9	±105.9	± 98.2	±100.4	±101.2	± 68.1 #
Severe	165.9	202.6	180.1	187.6	197.3	196.0	237.5
exercise(SD)	± 86.6	±119.9	± 95.0	± 96.7	±109.7	±114.6	±168.1 #

1. # : $p < 0.05$ in a comparison at each interval between different exercise.
2. There is no significant difference in paired comparison between value at start and at different intervals; also between different exercise, except in control group with severe exercise at the 24 hours later.

Table 4. Correlations of Exercise-induced Changes in Myoglobin, Uric Acid and Creatine Kinase

	MG versus UA	MG versus CK	UA versus CK
Experimental Group			
Moderate	r = 0.0389	r = 0.857	r = 0.2717
exercise	p>0.05	p<0.05	p>0.05
Severe	r = 0.8692	r = 0.528	r = 0.2898
exercise	p<0.05	p>0.05	p>0.05
Control Group			
Moderate	r = 0.0537	r = 0.7078	r = 0.2880
exercise	p>0.05	p<0.05	p>0.05
Severe	r = 0.7629	r = 0.2209	r = 0.1607
exercise	p<0.05	p>0.05	p>0.05

1. Abbreviation: MG = myoglobin, UA = uric acid, CK = creatine kinase
2. The comparison was performed between the data in each person through the course.

exercise ($p=0.0062$ in experimental group and $p=0.0011$ in control group).

The statistical data also indicated that the serum uric acid levels obtained within each group at various time interval of sampling, 0, 0.5, 1, 1.5, 2, and 24 hours, were significantly different ($p < 0.005$), but there was no significant differences between control and experimental groups that underwent the same extent of exercise versus the time intervals of sampling.

Serum Creatine Kinase

The results of serum creatine kinase levels before and after the course of exercise for both control and experimental groups designated for either moderate or severe exercise are listed in Table 3. Before the exercise, serum creatine kinase levels of 196.00 + 104.52 IU/L and 165.97 + 86.38 IU/L in control groups and 241.83 + 124.61 IU/L and 256.79 + 180.08 IU/L in experimental groups designated for moderate and severe exercise were obtained. Statistically, there was no significant difference.

The serum creatine kinase levels increased immediately following the exercise and declined within 30 minutes in both the control and the experimental groups after either severe or moderate exercise. In the control groups, a delayed, mild elevation of serum creatine kinase seemed present and persisted for 24 hours. However, the difference was not significant ($p > 0.05$) in either comparison.

There were higher values of serum creatine kinase levels in the experimental group than those in the control group. However, no significant difference is noted in statistical evaluation.

Correlation of changes of between myoglobin, uric acid and creatine kinase.

In statistical evaluation of the simultaneous, biochemical changes in each person through the follow-up course for 24 hours, the positive correlation ($r=0.857$ and 0.7078 respectively in experimental and control groups) was found between the elevations of myoglobin and creatine kinase in moderate exercise, but not in severe exercise. A positive correlation also existed between the changes of myoglobin and uric acid in severe exercise, but not in moderate exercise in both experimental group ($r=0.8692$) and control group ($r=0.7629$). There was no evident correlation between uric acid and creatine kinase in either comparison. (see Table 4)

DISCUSSION

The results of the current study indicate the rise of myoglobin, uric acid and creatine kinase after the exercise stress, and higher elevations were found in those with severe exercise in both control and experimental groups. In the experimental group, three of the fifteen (20%) volunteered subjects had serum myoglobin level over 90 ng/mL, and 4 of the 15 (26%) had the uric acid over 7.5 mg/dL, while in the control group, 1 (6.7%) had a serum myoglobin level over 90 ng/mL and 7 (46.7%) had uric acid over 7.5 mg/dL. These increases can not be attributed to either dehydration or the reduction in circulating blood volume during the test since a couple of parameters e.g. high density lipoprotein, low density protein, serum glutamic oxaloacetic aminotransaminase (GOT) showed little or no rise after the exercise (not listed).

Following the test run, the serum myoglobin levels were much higher in both control and experimental groups that underwent severe exercise than those underwent only the moderate exercise within the same group. One interesting observation is that the level of myoglobin in the experimental group is higher than its counterpart in the control

500 autoanalyzer.

Serum creatine kinase was determined on a Beckman Astra 8 autoanalyzer, and the analyses were made according to the recommendations of the manufacturer. The measurement involves the conversion of the substrates, creatine phosphate and adenosine-5'-diphosphate (ADP), to creatine and adenosine-5'-triphosphate (ATP) which is followed with coupled reactions with hexokinase and glucose-6-phosphate dehydrogenase in the presence of glucose and NAD. The conversion of NAD to NADH, which is stoichiometrio with the conversion of creatine phosphate to creatine, is proportional to the amount of creatine kinase in the sample, and can be determined spectrophotometrically at 340 nm.

RESULTS

Serum Myoglobin

The results of serum myoglobin levels before and after exercise in both control and experimental groups that designated moderate or severe exercises are listed in Table 1. A significant elevations of serum myoglobin levels is noted in both control and experimental groups that went through severe exercise. The peaks were reached in one hour after the start of the exercise and remained elevated for several hours. Serum myoglobin levels in both returned to the starting levels after 24 hours unless there were injuries involved. However, little or no change was observed for both control and experimental groups that went through moderate exercises.

There was a significant increase in the serum myoglobin levels in those who underwent severe exercise when compared with those who underwent only moderate exercise within the control group ($p < 0.05$). Similar difference was noted also within the experimental group ($p < 0.05$). The difference was more evident at one hour after the exercise (p# 0.0152 in experimental group, and p*0.0009 in control group by T-test).

Statistical analysis also revealed that the serum myoglobin levels obtained within each group at various time intervals, 0, 0.5, 1, 1.5, 2, and 24 hours, were significantly different ($p < 0.005$), but there was no significant difference between control and experimental groups that underwent same extent of exercise versus the time interval of sampling ($p > 0.05$).

Serum Uric Acid

The results of serum uric acid levels obtained before and after the course of exercise for both control and experimental groups designated for either severe or moderate exercise are listed in Table 2. Little or no change of the serum uric acid level was obtained for either control or experimental groups those underwent moderate exercise during the period of 24 hours of monitoring. However, the both groups when undergoing severe exercise showed significant increase in serum uric acid levels. The increase was observed immediately following the exercise, reached its peak within 30 minutes, and the elevation remained for 2 hours and 1.5 hours in experimental and control groups respectively; and returned to the original level when monitored 24 hours after the exercise.

In statistical analysis, a significant differences was detected between those underwent moderate exercise and those underwent severe exercise in both groups. The different became evident at 30 minutes after the exercise in the control group, and immediately after the exercise in the experimental group. The peak of difference was at one hour after the group. This most likely arises from the fact that the experimental group volunteers are well trained runners and are less likely to suffer from the possible muscle injury than the non-runners (7). Since myoglobin is a low molecular weight protein (mol. wt. 17,200), and is filtered by the renal glomeruli. It has been reported that low molecular weight substance like myoglobin can be reabsorbed through tubular system (8). Myoglobin does not bind to haptoglobin or any other plasma protein and thus may have a toxic effect on renal cells (9).

The peaking of serum myoglobin in one hour after the exercise as observed in both the control and the experimental groups was different from that reported by Thomas and Moffley (10). This difference may be due to variations in physical conditions as well as maximum oxygen uptake of the volunteers. Nevertheless, the myoglobin level returned to the starting level after 24 hours and is in good agreement with the reports of Demos et al (11).

The observation of the rise of serum uric acid levels in both experimental and control groups that underwent severe exercise and little or no change of serum uric acid level in those underwent moderate exercise are in good agreement with the observation of Green and Fraser (2). Moderate exercise apparently does not effect the uric acid level in well conditioned runners. Also the rise of the uric acid level is less in the trained runners when compared to the non-runners and is similar to the reports of Cooper and coworkers (12, 13).

Contrary to the reports of Benson et al (14), the serum creatine kinase level rises in 5 minutes after exercise and often rises above the normal values. Higher levels of creatine kinase were observed in the experimental group when compared to its counter part in the control group even the start value. This observation is similar to earlier reports (7, 15–19). However, the difference is not so significant in statistical consideration. The levels of creatine kinase increased 12% and 14% for the experimental groups designated for moderate and severe exercise, respectively; and increased 5.6% and 22% for the control groups designated for moderate and severe exercise, respectively. The reason of a high serum levels of creatine kinase in persons of regular exercise e.g. trained runner in this study is not well studied. The fact that a higher level of creatine kinase in control groups of severe exercise is found at 24 hours late may point out a possibility of muscle injury.

Besides, we also found a positive correlation existed between the simultaneous elevation of myoglobin and creatine kinase in moderate exercise but not in case of severe exercise, and between myoglobin and uric acid in severe exercise only in both groups. In our knowdge, there is no published data concering this fact. The phenomena and its possible mechaniam need further study.

In conclusion, both control and experimental groups have been found to sustain damage to skeletal muscle cells in all volunteers who underwent exercise, as verified by the increased in serum concentration of myoglobin and creatine kinase. Apparently, the myopathy in the serum during such exercise are quite common phenomena, in contrast to the earlier opinion which rest upon the less sensitive assay methods used (20). The results of the current study also suggests that creatine kinase is less sensitive than myoglobin in detecting changes after exercise stress and is consistent with the report of Maxwell and Bloor (7).

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專業跑者與非專業跑者於長賽跑後血中肌紅素尿酸、肌酸動力酶濃度之影響效應

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本研究係針對劇烈或中度等不同程度之運動對血清肌紅素，尿酸和肌酸動力酶之影響而言。十五位具最大氧攝取量（Max Vo2）大於或等於50ml／kg－min之專業長跑者為實驗組，十五位Max Vo2小於或等於45Ml／kg－min之非專業跑者為對照組。（兩組之年齡均界於17－29且歲且均為男性）

在實驗組與對照組之劇烈運動後，血清肌紅素呈有意義之上升，但中度運動後卻無此現象。實驗組上升程度較高，而兩組之肌紅素均於運動後1小時內上升；24小時內回復正常。

尿酸情形類似肌紅素之變化，但係在運動後30分內上升，24小時內回復正常。但尿酸不同於肌紅素在於對照組上升程度大於實驗組。

肌酸動力酶在兩組之劇烈運動後呈輕微而非有意義之上升，而每組中之劇烈或中度運動後之動力酶增加，無有意義之差別。

我們試圖發覺此三者間之相關變化，而發現肌紅素與尿酸在劇烈運動時呈正相關，且肌紅素與肌酸動力酶在中度運動時則反之。

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