Turk. J. Med. Sci.
26 (1996) 21-25
© TÜBİTAK

Fadıl ÖZYENER
Hakan GÜR
Kasım ÖZLÜK

Changes in Fibrinolytic Activity Following Brief Exhaustive Maximal Exercise in Sedentary Males

Received: October 27, 1994

Department of Physiology and Sports Medicine, Medical Faculty of Uludağ University, Bursa Turkey

Abstract: Changes in fibrinolytic activity (FA) were investigated and followed for 4 hours after brief exhaustive maximal exercise in sedentary males. Sixteen volunteer, healthy, nonsmoking subjects aged 23.0±3.9 (19-30) performed a maximal cycling exercise to exhaustion. Euglobulin lysis time (ELT), fibrin plate lysis area (FPLA), fibrinogen (F), tissue-type plasminogen activator (tPA), pH and lactate (L) concentrations in plasma were measured before, immediately, and one and four hours after the exercise. The results of FPLA and tPA increased immediately ($p<0.001$) and 1 hour after the exercise, whereas they decreased at the 4th hour of the revovery period. ELT decreased immediately and 1 hour after the exercise ($p<0.001$) and increased at the 4th hour of the recovery period. However, F values did not change significantly during the same period. Thus FA increased following brief exhaustive maximal exercise and continued to increase 1 hour after the completion of exercise, but it was inhibited in the late recovery period.

Key Words: Maximal exercise, fibrinolysis, sedentariness recovery period

Introduction

There is a general agreement that physical exercise performed regularly prevents cardiovascular diseases. However, it has also been reported that strenuous physical exercise can be the cause of sudden cardiovascular death due to increased thrombosis (1-3). It is very well established that acute exercise leads to a temporary increase in fibrinolytic activity (FA) depending on the intensity and duration of exercise (2, 4-11). Increasing tissue (tPA) (11, 12) and urokinase-type (u-PA) (13) plasminogen activator levels in plasma have been claimed to be responsible for the increase in FA. It has also been suggested that plasminogen activator inhibitor type 1 (PAI-1) is one of the prominent regulators of FA (5, 6, 14). On the other hand, an increase in FA has been attributed to a cellular component, possibly because of the fibrinolytic enzymes being relased from neutrophilic granulocytes (15). In addition, increasing levels of cateocholamines and exercise-produced metabolites are proposed to be responsible for the changes in FA due to stimulated tPA release from the vascular endothelium (11, 16).

The changes in FA during and immediately after exercise, and the probable mechanisms of these changes have been very well established by several authors. Nevertheless, there is a lack of knowledge about the changes in FA during the recovery period. While it has been reported by some authors that increased FA after exercise returns to the pre-exercise levels 30-60 minutes later (17), some researchers found that the increment continues for hours and hours after the completion of exercise (18, 19) and some others observed that it is inhibited in 2-4 hours of the recovery period (20).

We examined the changes in FA following brief exhaustive exercise in sedentary males and followed the changes in FA for 4 hours during the recovery period.

Materials and Methods

Subjects: Sixteen healthy, non-smoking, sedentary male volunteers (mean age 23, range 19-30) were included in the study. Physical characteristics of the subjects are summarized in Table 1. All subjects were informed about the procedures and risks of the study and completed an extensive medical questionnaire about family medical history. All subjects also underwent a medical examination before the experiments

Table 1. Physical Characteristics of Subjects (n=16).

	Mean	SD	Min-Max
Age (yr)	23.0	3.9	19-30
Height (cm)	177.2	4.7	170-183
Weight (kg)	70.2	9.0	61-88
Body fat (%)	15.5	2.8	11.1-18.9
VO_2 max	42.2	3.8	36.3-51.5
($ml.kg^{-1}$ dak^{-1})			
VO_2 max	2.49	0.38	2.09-3.45
($L.dak^{-1}$)			
Respiratory Quotient (RQ)	1.13	0.04	1.1-1.2
Maximal Heart Rate (min)	191	5	185-201

and were declared fit for the maximal exercise. Each subject performed a submaximal and maximal test before the performance test to determine the maximal work load and its percenatges for the performance test. Percentage of body fat (21) and surface area (22) were calculated for each subject. Heart rates were monitored by a pulse recoder (OxyShuttle, Sensormedic USA) throughout the tests.

Exercise Protocols: The exercise tests were performed on a bicycle ergometer (Monark Weight Ergometer 814 E, Sweden), pedaling at 60 rpm. This was calibrated by controlling the connection between the pedaling rate and the load prior to each test. All subjects were familiarized with the bicycle ergometer before the tests.

Submaximal Test: The subjects performed a test involving 4 stages and lasting 16 minutes. Load was increased gradually every 4 min. according to the heart rate reaching 100, 120, 140 and 160±5 beats/min at the end of the 1st, 2nd, 3rd and 4th stages, respectively. At the second min of every stage, the subjects were asked to point out the intensity of exercise according to Borg's scale (23).

Maximal Test: Initial load for the test was chosen according to submaximal test such that subjects became exhausted in 7 to 10 min. The load was increased subsequently in three-minute intervals until subjects informed that they could not continue. The maximal test attainment criteria included the plateau in oxygen consumption in spite of work load increase, respiratory exchange ratio greater than 1.15 and no further increase in heart rate.

A regression analysis between the work loads applied in submaximal tests and the average oxygen consumption was performed for each subject. Therefore, 50 and 80% of the maximal load were calculated from regression equations for each subjects.

Performance Test: The subjects arrived in the laboratory in the early morning after a light breakfast. Before starting the performance test, a 4-minute warm-up period, including 3 and 1 min. of cycling at 50 and 80% of the subjects' maximal load, respectively, were allowed. After a three-minute rest following the warm up, each subject cycled at their maximal load until exhaustion. During the maximal and performance test, all subjects were encouraged to reach their maximal effort. Following the performance test, subjects were seated and were allowed to have liquids but not to eat. During the recovery period, subjects were allowed to conduct minimal activites such as reading, etc.

Metabolic Analysis: During the submaximal and maximal tests, subjects were connected to a metabolic analyzer (Sensormedics 2900 C, The Netherlands) with a two-way, mouth-face mask and a tube system (NRVD-T shape, Hans Rudolf Inc., USA). Subjects breathed through the mask and the oxygen consumption, carbon dioxide production and ventilation volume were calculated breath by breath throughout the submaximal and maximal tests. The results were corrected according to temperature, barometric pressure, and humidity of the laboratory.

Blood Samples: Blood samples were drawn from the cubital vein of the forearm via a cannula (Vasofix 18G, Germany) which had a three-way tap (Veinflex, Denmark) before, immediately, one hour, and four hours after the exercise. Blood samples were collected into plastic tubes containing 3.8 % sodium citrate in a 9: 1 (v:v) ratio. Plasma was separated immediately by centrifugation (1.200 x g for 10 min) and stored at -20°C until further testing within three weeks maximum. The cannulas were heparinized after taking each blood sample and the first 2-3 ml of samples were discarded to eliminate the efect of heparin. The plasma volume changes were determined according to the Van Beaumot formula (24) and all results were corrected for changes in plasma volume.

Hematological Analysis: Euglobilin Lysis Time (ELT) was determined according to Nordby et al. (25). Determination of Fibrin Plate Lysis Area (FPLA) in plasma was carried out acording to Astrup et al. (26). Plasma fibrinogen (F) levels were measured as explained by Bowie et al. (27). tPA plasma levels were determined using a commercial Assechrom-tPA ELISA-kit obtained from Sigma, France by ELISA assay (28).

ELT, FPLA and F values were determined in fresh plasma. A total of 5 ml of the plasma was stored at -20°C for tPA analysis within the next three weeks.

Statistics: Linear regression analysis was used to determine the relationship between oxygen consumption and work load. ANOVA for repeatad measures was performed to analyze variation in the four blood samples. When the analysis indicated the presence of a significant difference, the mean were compared by the student's t-test for paired data. Values less than 0.05 were considered to be significant. All values have been presented as mean±SD.

Results

The subjects (n= 16) performed 7.08±1.56 min (4.31-10.40) exercise until exhaustion at their maximal work loads. With the exercise, plasma lactate and pH levels changed from 2.2±0.3 mM/L and 7.36±0.03 to 15.6±2.6 mM/L and 7.18±0.08, respectively (Table 2).

Table 2. The Results (n= 16) of Lactate (L) pH, Euglobulin Lysis Time (ELT), Fibrin Plate Lysis Area (FPLA), Fibrinogen (F) and Tissue Type-Plasminogen Activator (tPA) in Plasma Before (BE), Immediately (IAE), 1 (1AE) and Four Hours (4AE) After the Exercise. Values are Mean±SD.

	BE 1	IAE 2	1AE 3	4AE 4	P<
L (mMM/L)	2.2±0.3 ^{2,3}	15.6±2.6 ^{1,3,4}	3.9±1.0 ^{1,2,4}	2.4±0.6 ^{2,3}	0.001
pH	7.36±0.03 ^{2,4}	7.18±0.08 ^{1,3,4}	7.37±0.03 ^{2}	7.38±0.02 ^{1,2}	0.001
ELT (min)	134±16 ^{2,3}	75±20 ^{1,3,4}	113±24 ^{1,2,4}	146±22 ^{2,3}	0.001
FPLA (mm^2)	82±35 ^{2,4}	273±125 ^{1,3,4}	101±41 ^{2,4}	59±25 ^{1,2,3}	0.001
F (mg/dl)	433±45	404±75	453±58	461±65	n.s.
tPA (ng/ml)	3.6±0.9 ^{2}	10.3±1.1 ^{1,4}	4.7±1.0 ^{4}	3.0±0.5 ^{2,3}	0.001

*p< value indicates the level of significance obtained in the analysis of variance (ANOVA). Superscript figures indicate differences between the four different times.

Euglobilin Lysis Time (ELT): ELT was 75±20 min. immediately after the exercise with a 44% drop (p<0.001) from the pre-exercise level; and it was elevated to 113±24 min. at the 1st hour of the recovery period, but was still 14% lower (p<0.001) than the pre-exercise level (Table 2, Figure 1). It continued to rise until the 4th hour of the recovery period and it reached a level which was 9 % higher than the pre-exercise level (Table 2, Figure 1)

Fibrin Plate Lysis Area (FPLA): FPLA was 82±35 mm^2 prior to exercise and reached 273±125 mm^2, a 233 % increment (p<0.001) immediately after the exercise. Although, it decreased to 101±41 mm^2 at the 1st hour of the recovery period, it was still 23 % lower than the pre-exercise level (Table 2, Figure 1).

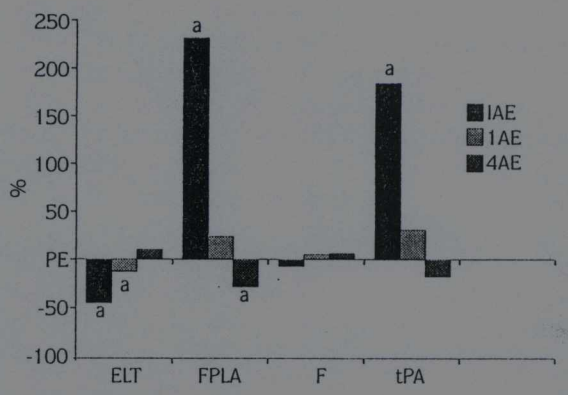

Figure 1. Comparison (%) of Euglobulin lysis Time (ELT), Fibrin Plate Lysis Area (FPLA), Fibrinogen (F) and Tissue Type-Plasminogen Activator (tPA) Values Determined in Plasma Immediately (IAE), 1 (1AE) and 4 Hours (4AE) After Exercise with Pre-Exercise Values (PE). "a" Denotes p<0.001 Compared to PE Values.

Four hours after exercise, FPLA decreased to 59±25mm^2 and reached a level 28% lower (p<0.001) than the pre-exercise level (Table 2, Figure 1).

Plasma Fibrinogen (F): F was determined to be 433±45 mg/dl before the exercise, dropped 7% immediately after exercise, and reached a level of 453±58 mg/dl at the 1st hour of the recovery period with a slight increment (Table 2, Figure 1). It showed a similar increment trend until the 4th hour of the recovery period, but the levels at the 1st and 4th hour of the recovery period were not significantly different from the pre-exercise level.

Plasma tissue type-Plasminogen Activator (tPA): tPA level in plasma was 3.6 ±0.9 ng/ml prior to exercise and it increased to 10.3±1.1 ng/ml (p<0.001) with 186% increment immediately after the exercise. Although, it was found to be decreasad to 4.7±1.0 ng/ml, it was still 31% higher than the pre-exercise level at the 1st hour of the recovery period (Table 2, Figure 1). It subsequently decreased and reached 3.0±0.5 ng/ml at the 4th hour of the recovery period and the level was 16% lower compared to the pre-exercise level (Table 2, Figure 1).

Discussion

The increase in FA following a brief exhaustive maximal exercise observed in the present study is in accordance with the results of previous studies (7-10). This increase might be related to an increased release of tPA (18,19, 29, 30). However, Speiser et al. (31)

suggested that the increase in FA immediately after exercise was due rather to a reduction in the PAI-1 level, and they reported that tPA activity was less responsible for the changes in FA. Controversially, Chandler et al. (11) observed that there was no change in PAI-1 level following maximal exercise whereas the tPA level increased relative to the plasma adrenaline level. It has been suggested that increases FA might be related to the percentage of the individuals' maximal aerobic capacity (8) and tha FA increases to a larger extent in response to maximal exercise in highly trained men (32). In addition, it has been pointed out that the level of acceleration of FA was related to the work load attained (29). In light of these suggestions, it can be concluded that the disagreement between these results might be due to the differences in intensity and duration of exercise and the training backgrounds of subjects used in these studies.

The results of ELT, FPLA and tPA at the 1st hour of the recovery period indicate that the increase in FA continues. Speiser et al. (31) also found that increased FA following maximal exercise performed by athletes and sedentary subjects continues at the 1st hour of the recovery period. Controversially, it was reported that increased FA after a middle-distance run continues at 30 min. and reaches a basal level at 60 min. (20). In addition, Dufaux et al. (17) have reported that plasma tPA level increases following maximal exercise performed by moderately trained individuals and returns to pre-exercise levels at the 30th and 60th min. Because it has been indicated that the response of the fibrinolytic system to acute exercise shows apparently different mechanisms associated with the physical conditioning of individuals (29), it seems that the differences between the results of these studies are due to the physical backgrounds of subjects.

The results (elongation in ELT, reduction in FPLA and decrease in plasma tPA level) 4 hours after the completion of exercise indicate an inhibiton in FA contrary to the results showing an increase in FA immediately and 1 hour after the exercise. The cause of inhibition observed in FA might be the reduction in plasma tPA level. However, although Hansen et al. (20) observed a reduction in FA and an elongation in whole blood clot lysis time, they have found that tPA level was at the basal level 2-4 hours after three different distance runs at maximal speed. Therefore, they suggest that a delayed and excessive release of PAI-1 from the endothelium or other inhibitors may be important in the inhibition of FA late in the recovery period.

In conclusion, the result of this study indicate that fibrinolytic activity increases following brief exhaustive maximal exercise and continues 1 hour after the completion of exercise, but it is inhibited in the late recovery period after exercise.

References

1. Amsterdam EA. Sudden death during exercise. Cardiol 77: 411-7, 1990.
2. Drory Y, Kramer MR, Lev B. Exertional sudden death in soldiers. Med Sci Sports Exerc 23: 147-51, 1991.
3. Kohl HW, Powell KE, Gordon NF, Blair SN, Paffenbarger RS. Physical activity, physical fitness, and sudden cardiac death. Epidemiol Rev 14: 37-57, 1992.
4. Colwell JA. Effects of exercise on platelet function, coagulation, and fibrinolysis. Diabet Metabol Rev 1: 501-12, 1986.
5. Sinzinger H, Virgolini I. Effects of exercise on paramaters of blood coagulaton, platelet function and the prostaglandin system. Sports Med 6: 238-45, 1988.
6. Bourney RE, Santoro SA. Intreractions of exercise, coagulation, platelets, and fibrinolysis: A brief review. Med Sci Sports Exerc 20: 439-46, 1976
7. Cash JD, Allan AGE. the fibrinolytic response to moderate exercise and intravenous adrenaline in the same subjects. Br J Haematol 13: 376-83, 1967.
8. Davis GL, Abildgaard CF, Bernauer EM, Britton M. Fibrinolytic and hemostatic changes during and after maximal exercise in males. J App Physiol 40: 287-92, 1976
9. Wheeler ME, Davis LG, Gillespie WJ, Bern MM. Physiological changes in hemostasis associated with acute exercise. J Appl Physiol 60: 986-90, 1986.
10. Drygas W. Changes in blood platelet function, coagulation, and fibrionlytic activity in response to moderate, exhausive, and prolonged exercise. Int J Sports Med 9: 67-72, 1988.
11. Chandler WL, Veith RC, Fellingham GW, Levy WC, Schwartz RS, Cerqueira MD, Kahn SE, Larson VG,Can K, Beard JC, Abrass IB, Stratton JR. Fibrinolytic response during exercise and epinephrine infusion in the same subjects. J Am Coll Cardiol 19: 1412-20, 1992.
12. Collen D, Lijnen HR. Basic and clinical aspects of fibrinolysis and thrombolysis. Blood 78: 3114-24, 1991.
13. Dooijewaard G, de Boer A, Turion PNC, Cohen AF, Brimer DD, Kluft C. Physical exercise induces enhancement of urokinase-type plasminogen activator (u-PA) levels in plasma. Thromb Haemostas 65: 82-6, 1991.

14. Sakata Y, Okada M, Noro A, Matsuda M. Interaction of tissue-type plasminogen activator and plasminogen activator inhibitor-1 on the surface of endothelial cells. J Biol Chem 263: 1960-9, 1987.

15. Langleben D, Moroz LA. Participation of blood cells in exercise-induced fibrinolysis. Thromb Res. 31: 733-40, 1985.

16. Hawkey CM, Britton BJ, Wood WG, Peele M, Irving MH. Changes in blood catecholamine levels and blood coagulation and fibrinolytic activity in response to graded exercise in man. Br J Haematol 29: 377-84, 1975.

17. Dufaux B, Order U, Liesen H. Effect of a short maximal physical exercise on coagulation, fibriolysis, and complement system. Int J Sports Med 12 (Suppl. 11): S38-S42, 1991.

18. Röcker L, Taenzer M, Drygas WK, Lill H, Heyduck B, Altenkirch HU. Effect of prolonged physical exercise on the fibrinolytic system. Eur J Appl Physiol 60: 478 81, 1990.

19. Arai M, Yorifuji H, Ikemaisu S, Fujimaki M, Fukutake K, Katsumara K, Ishii T, Iwane H. influences of strenuous exercise (triathlon) on blood coagulation and fibrinolytic system . Thromb Res 57: 465-71, 1990.

20. Hansen JB, Wilsgard L, Olsen JO, Osterud B. Formation and persistence of procoagulant and fibrinolytiic activites in circulation after strenous physical exercise. Thromb Haemostas 64: 385-9, 1990.

21. Womersly J, Durnin IUGA. Body fat assessed from total bdy density and its estimation from skin fold thickness: Measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 32: 77-9, 1974.

22. Boothby WM, Sandiford RB. Nomographic charge for computing body surface area. In: The physiological basis for exercise and sport (Eds. E Fox). Brown & Benchmark, Iowa, pp 647-50, 1993.

23. Borg GAV: Perceived exertion: Quote on history and methods. Med Sci Sports 5: 90-3, 1973.

24. Van Beaumont W: Evalutaion of hemaconcentration from hematocrit measurements. J Appl Physiol 32: 712-3, 1972.

25. Nordby E, Arnesen H, Andersen P. The euglobulin clot lysis time, a rapid and sensitive method for the assay of fibrinolytic activity after venous stasis. Scand J Haematol 25: 407-11, 1980.

26. Astrup T, Müllertz S. The fibrin plate method for estimating fibrinolytic activity. Arch Biochem 40: 346, 1952.

27. Bowie EJ, Thompson JH, Didisheim P. Laboratory manual of hemostasia. W.B. Saunders, Philedelphia, pp. 95, 1971.

28. Holvoet P, Cleemput H, Collen D.Assay of human tisue-type plasminogen activator (tPA) with a enzyme linked immunostorbent assay (Elisa) based on three murine monoclonal antibodies to tPA. Throm Haemost 54: 684-7, 1985.

29. De Paz JA,Lasierra J, Vilades E, Martin-Nuno MA, Gallego JG. Changes in the fibrinolytic system associated with physical conditoning. Eur J Appl Physiol 65: 388-93, 1992.

30. Huisveld IA, Kluft C, Hospers AJH, Bernink MJE, Erick WBM, Bouma BN. Effect of exercise and oral contraceptive agents on fibrinolytic potential in trained females. J Appl Physiol 56: 906-13, 1984.

31. Spesier W, Langer W, Pschaick A, Semyar E, Ibe B, Nowacki PE, Muller Berghaus. Increased blood fibrinolytic activity after physical exercise: Comparative study in individuals with different sporting activites and in patients after myocardial infarction taking in a rehablitation sports program. Thromb Res 51: 543-55, 1988.

32. Ferguson EW, Bernier LL, Banta GR, Yu-Yahiro J, Schoomaker EB. Effects of exercise and conditioning on clotting and fibrinolytic activity in men. J Appl Physsiol 62: 1416-21, 1987.