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Fibrinolytic Responses to Moderate Intensity Exercise

Comparison of Physically Active and Inactive Men

Linda M. Szymanski, Russell R. Pate

Abstract The purposes of this study were to compare fibrinolytic responses to moderate intensity exercise in physically active and inactive men and during morning and evening exercise. Fourteen physically inactive men (mean age, 34.7±4.0 years) and 12 regularly active men (34.8±4.0 years) performed two exercise sessions, morning and evening, at 50% of maximal oxygen consumption. Tissue plasminogen activator (TPA) and plasminogen activator inhibitor-1 (PAI-1) activity were measured before and after exercise. Data were analyzed using a three-way ANOVA with repeated measures. TPA activity increased with exercise in both groups, although the active group demonstrated greater increases than the inactive group. Postexercise TPA activity was greater with evening than morning exercise. The inactive group exhibited greater PAI-1 activity than the active group. PAI-1 activity was higher during the morning than evening but did not change with exercise for either group. We conclude that moderate intensity exercise increases TPA activity in physically active and inactive men, with greater increases seen in active men, particularly during evening exercise. Moderate intensity exercise does not appear to affect PAI-1 activity. The lower PAI-1 activity in active men may be one mechanism whereby regular physical activity lowers the risk for coronary artery disease. (*Arterioscler Thromb.* 1994;14:1746-1750.)

Key Words • diurnal variations • fibrinolysis • physical fitness • tissue plasminogen activator • plasminogen activator inhibitor

Enhanced fibrinolytic activity is often listed as a benefit of regular participation in physical exercise; however, little is known about the fibrinolytic responses to exercise and potential differences that may exist between physically active and inactive individuals. Although it is well documented that short-term physical exercise increases fibrinolytic activity,[1-5] most studies describe global fibrinolytic activity and have not measured the fibrinolytic components tissue plasminogen activator (TPA) and its inhibitor plasminogen activator inhibitor-1 (PAI-1). In addition, few studies that examined fibrinolytic responses to exercise have controlled for the physical activity status of subjects. Studies measuring global fibrinolytic activity[5] and the fibrinolytic components[6] report that the activity status of subjects significantly influences the magnitude of change in fibrinolytic variables in response to maximal exercise. Furthermore, few studies have described the responses to a typical submaximal exercise session.

Resting fibrinolytic activity is lowest in the morning and increases throughout the day.[7-11] Lower morning values have been attributed to low TPA activity and high PAI-1 activity.[7,12] These resting diurnal variations appear to affect the fibrinolytic response to exercise. Data from our laboratory[13] suggest that evening exercise produces greater increases in TPA activity than morning exercise. We are aware of only one other study that has examined the effects of exercise performed at different times of day on the fibrinolytic response to exercise. Rosing et al[10] found significantly greater global fibrinolytic activity after evening exercise compared with morning exercise. However, only two subjects were tested, making it difficult for one to draw firm conclusions from their data.

The purposes of the present investigation were twofold. First, we compared fibrinolytic responses to a moderate intensity exercise session in physically active and inactive men. Second, we examined fibrinolytic responses during morning and evening exercise. We measured TPA and PAI-1 activities to assess fibrinolytic activity.

Methods

Subjects

Subjects were 26 apparently healthy adult men 28 to 43 years of age. All subjects were nonsmokers, nondiabetic, nonobese, and had no evidence of type IV hyperlipoproteinemia. Subjects were not currently taking any medications, including aspirin. Inactive subjects (n=14) had not participated in regular physical activity for at least the previous 3 months. Regularly active subjects (n=12) had participated in regular physical activity (jogging) for approximately 30 minutes per session 3 to 5 d/wk for the previous 3 months or more. All subjects volunteered to take part in the study and gave written informed consent before participation. The protocol was approved by the university's Institutional Review Board.

Design

All subjects underwent a maximal graded treadmill exercise test to determine their maximal oxygen consumption ($\dot{V}O_2max$) and then on separate days performed two 30-minute submaximal exercise sessions on the treadmill. The submaximal sessions were at 50% of their $\dot{V}O_2max$, one in the morning and one in the evening. The order of the sessions was

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randomized and counterbalanced. All morning testing was conducted between 6:30 and 10 AM, and evening testing was conducted between 4 and 7 PM with at least 2 days separating sessions. Subjects reported to the morning testing session in a fasted state (12 hours) and were instructed not to engage in physical activity at least 24 hours before a testing session, to refrain from ingesting aspirin and nonsteroidal anti-inflammatory drugs 14 days before a testing session, and to maintain similar eating patterns throughout the study. Instructions before an evening session were to refrain from eating at least 3 hours before the testing session and to refrain from ingesting caffeine after 10 AM.

Blood Collection

Blood was collected before and immediately after exercise with subjects in the seated position. Preexercise samples were obtained after 10 minutes of sitting rest. Blood samples were drawn by venipuncture from an antecubital vein with little or no stasis into 5-mL vials containing 50 μL of 15% K_3-EDTA. The first vial was used for hematocrit and hemoglobin determinations. The second vial was used for TPA and PAI-1 determinations. Anticoagulated blood was combined 2:1 with 0.5 mol/L sodium acetate (pH 4.2) within 60 seconds of being drawn to stabilize TPA activity. Blood was centrifuged at 1000g for 10 minutes at room temperature. Plasma was separated and stored at −80°C until analyzed.

Experimental Conditions

Maximal Graded Exercise Test

A modified Balke treadmill exercise protocol[14] designed to fatigue all subjects within 11 to 15 minutes was used. Heart rate was monitored via electrocardiography. Oxygen consumption was continuously monitored by an automated system (Rayfield Equipment) using an Applied Electrochemistry S-3A O_2 analyzer (Ametek), a Beckman LB-2 carbon dioxide analyzer, and a Parkinson-Cowan gasometer. $\dot{V}O_2$max was defined as the highest oxygen consumption observed during any full minute of the exercise test. The criteria used for attaining $\dot{V}O_2$max included a plateau of oxygen consumption with an increasing work rate, a respiratory exchange ratio ≥1.05, and/or a maximal heart rate within 5 beats per minute of age-predicted maximum.

Submaximal Exercise Sessions

The two submaximal sessions were performed on the treadmill for 30 minutes. Workloads were determined using the heart rate and oxygen consumption data from the maximal exercise test. To ensure subjects were exercising at appropriate intensities, oxygen consumption was monitored for 5 to 10 minutes during each exercise session.

Blood Analyses

Hematocrit and Hemoglobin

Hematocrit was measured in triplicate using the standard microhematocrit technique. Hemoglobin concentration was assayed in duplicate using the cyanmethemoglobin method.[15] Percent changes in plasma volumes were estimated from the hematocrit and hemoglobin values.[16]

TPA Activity

TPA activity (expressed in international units [IU]) was measured by chromogenic assay under the optimal conditions as described by Chandler et al.[17] Briefly, 5 μL anticoagulated, acidified blood was added to 250 μL plasminogen-chromogenic substrate reagent consisting of 75 mmol/L Tris–acetic acid (pH 8.15 at 37°C), 0.1% Triton X-100, 0.50 μmol/L human Glu-plasminogen (American Diagnostica), 0.65 mmol/L S-2251 substrate (Kabi Diagnostica), and 80 μg/mL CNBr-cleaved fibrinogen and was incubated at 37°C for 90 minutes. After incubation, the reaction was stopped by addition of 25% acetic acid to the solution. Absorbance was measured at 405 nm. A standard curve made with one-chain melanoma-derived TPA (American Diagnostica) was developed to determine TPA activity. Results were multiplied by 1.5 to correct for acetate buffer dilution and were also corrected for changes in plasma volume.

PAI-1 Activity

PAI-1 activity (expressed in arbitrary units [AU]) was measured by chromogenic assay according to the standardized method of Chandler et al.[18] Briefly, plasma was diluted 1:2, 1:5, 1:10, and 1:20 with phosphate-buffered saline (PBS)/Triton X buffer containing 1 g bovine albumin and 0.6 mmol sodium azide per liter. Then, 200 μL of the diluted plasma was mixed with 200 μL of 10 IU/mL one-chain TPA reagent (diluted in PBS/Triton X buffer) and incubated for 15 minutes at 37°C to allow TPA and PAI-1 to react. The reaction was stopped and any α_2-plasmin inhibitor was destroyed by addition of 200 μL of 0.5 mol/L sodium acetate buffer (pH 4.2) to the solution. Residual TPA was measured as described above. Since plasma dilutions that inhibit less than 8% and more than 50% of the original TPA have produced inaccurate results,[18] only dilutions that inhibited between 8% and 50% of the original TPA were used to determine residual TPA activity. One AU of PAI-1 activity was defined as the amount of PAI-1 that inhibited one IU of TPA under the specified conditions. Results were corrected for changes in plasma volume.

Samples from each subject were analyzed at one time to control for interassay variations. For determination of interassay variation for the fibrinolytic variables, blood drawn from a single individual was stored in aliquots and assayed with each batch.

Other Measures

Height, weight, and skinfold thicknesses were measured on all subjects. Body fat was determined using four sites (abdomen, ilium, tricep, thigh) according to equations by Jackson and Pollock.[19] To screen for hypercholesterolemia and hyperlipoproteinemia, total cholesterol, high-density lipoprotein cholesterol, and triglyceride concentrations were measured before participation (Abbott Vision). Blood was drawn in the morning with subjects in a fasted state (12 hours).

Statistical Analyses

One-way ANOVA was used to compare demographic and descriptive variables between the groups. TPA and PAI-1 activities were analyzed before and after submaximal exercise using a 2 (group) ×2 (time of day, morning versus evening) ×2 (time, before versus after) ANOVA with repeated measures. Least-squares means were computed to make preplanned comparisons. Statistical significance was reached at a value of $P<.05$.

Results

Descriptive Characteristics

Table 1 summarizes descriptive characteristics of the subjects. As expected, the regularly active men had a significantly lower percentage of body fat and attained a significantly higher $\dot{V}O_2$max than the inactive men. Table 2 summarizes results from the exercise sessions. The actual oxygen consumption measured during submaximal exercise sessions closely approximated 50% of maximum for both groups for both sessions.

TPA Activity

Fig 1 presents results for TPA activity (IU per milliliter) corrected for estimated changes in plasma volume before and after the submaximal exercise sessions. ANOVA found significant main effects for time of

TABLE 1. Descriptive Characteristics of Subjects

Variable	Inactive (n=14)	Active (n=12)
Age, y	34.7±4.0	34.8±4.0
Weight, kg	83.5±16.6	79.8±10.3
Body mass index, kg/m^2	26.2±4.8	24.5±2.7
Body fat, %	21.3±5.1	16.5±5.8*
Total cholesterol, mmol/L	4.79±0.98	4.14±1.00
HDL cholesterol, mmol/L	1.07±0.28	1.18±0.25
Triglyceride, mmol/L	1.48±1.07	0.98±1.37
$\dot{V}o_2$max, mL · kg^{-1} · min^{-1}	38.6±5.6	51.4±3.9*

HDL indicates high-density lipoprotein. Values are mean±SD.
*P<.05 between groups.

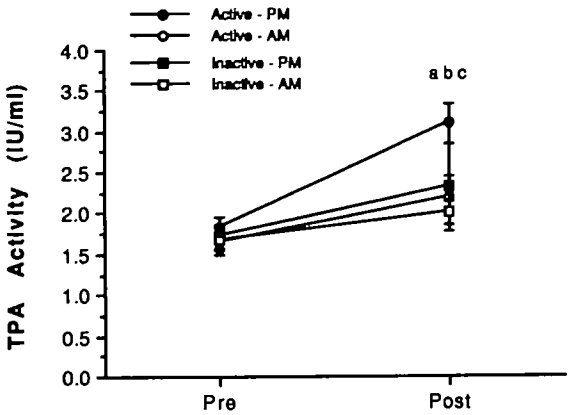

FIG 1. Line graph shows tissue plasminogen activator (TPA) activity before and after exercise in physically active and inactive men. Exercise was performed at 50% $\dot{V}o_2$max during the morning and evening. Values are mean±SEM. a indicates postexercise value different from preexercise (P<.05); b, different AM and PM values (P<.05); and c, PM value in active group higher than other values P<.05).

day (P<.005) and time (P<.001). Larger increases in TPA activity were observed after evening exercise than morning exercise, and postexercise values were greater than preexercise values. Although the active group had slightly higher preexercise TPA activity, this difference was not statistically significant. Exercise produced significant increases in TPA activity in both groups for both sessions. Postexercise TPA activity in the active group for the evening session was significantly higher than all other postexercise values.

PAI-1 Activity

Fig 2 shows results for PAI-1 activity (AU per milliliter) corrected for estimated changes in plasma volume. ANOVA indicated significant main effects for group (P<.005) and time of day (P<.05). Higher PAI-1 activity values were observed in the inactive group compared with the active group, and PAI-1 activity was higher in the morning than in the evening. Preexercise PAI-1 activity was higher in the morning sessions compared with evening sessions, but this was significant in the inactive group only. PAI-1 activity did not change with exercise in either group for either session (P>.05).

Intra-assay and Interassay Variations

The intra-assay and interassay coefficients of variation for TPA were 4.7% and 5.6%, respectively, and for PAI-1 activity were 2.5% and 5.7%, respectively.

Discussion

This investigation compared the fibrinolytic responses to moderate intensity physical exercise in habitually physically active and inactive men and also compared the responses to exercise performed in the morning and evening. The major findings were that moderate intensity exercise increased TPA activity in physically active and inactive men regardless of whether the exercise was performed in the morning or evening and greater increases were observed in the active group than the inactive group. Additionally, postexercise TPA activity was greater with evening exercise than morning exercise. PAI-1 activity was significantly higher in the inactive group and did not change significantly with exercise in either group.

There is some disagreement in the literature regarding the effect of activity status or fitness status on fibrinolytic activity. Cross-sectional studies examining resting global fibrinolysis in active and inactive individuals report conflicting results. Higher fibrinolytic activity has been reported in active individuals,[20] but not all studies agree with this finding.[2,5] Few studies have measured the fibrinolytic components TPA and PAI-1. However, greater global fibrinolytic activity[5] and greater increases in TPA activity[6] have been reported after exercise in active individuals compared with their inactive counterparts. Exercise training studies[21,22] have also reported beneficial changes in both resting TPA and PAI-1 activity; however, not all study designs included control groups, making it difficult to confidently attribute the observed changes solely to the training program.

The mechanisms responsible for increased fibrinolytic activity with short- and long-term exercise have not yet been completely resolved. Recent investigations by Chandler et al[23,24] demonstrate that TPA levels in the

TABLE 2. Submaximal Exercise Results

	Submaximal Exercise Sessions			
	Inactive		Active	
Variable	50% AM	50% PM	50% AM	50% PM
50% $\dot{V}o_2$max, mL · kg^{-1} · min^{-1}	19.3±3.3	19.6±3.1	25.9±3.0	25.8±2.7
Respiratory exchange ratio	0.89±0.03	0.90±0.02	0.88±0.02	0.91±0.03
Caloric expenditure, kcal	233±34	239±36	304±37	303±48

Values are mean±SD.

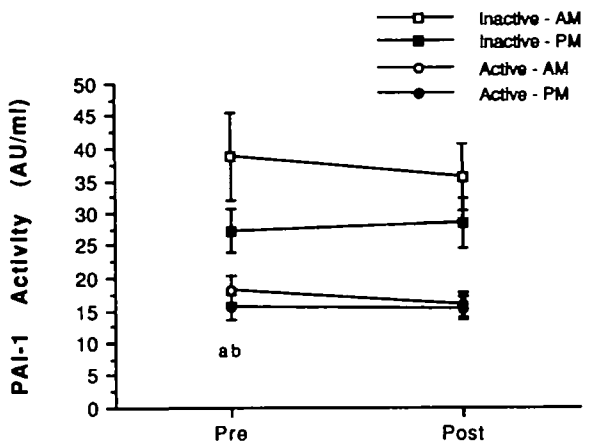

FIG 2. Line graph shows plasminogen activator inhibitor-1 (PAI-1) activity before and after exercise in physically active and inactive men. Exercise was performed at 50% Vo_2max during the morning and evening. Values are mean±SEM. a indicates inactive and active groups are different ($P<.05$); b, AM and PM values are different in inactive group ($P<.05$).

blood are directly proportional to plasma epinephrine concentrations. deBoer et al[25] found increases in TPA during exercise to be related to reductions in liver blood flow, suggesting that hepatic clearance of TPA also plays a role. Additional research is needed to determine other potential mechanisms that may account for the increases in TPA with short-term exercise that are not explained by epinephrine stimulation and hepatic clearance.

Mechanisms explaining enhanced fibrinolytic activity as a possible adaptation to regular physical activity are also not known but may be related to enhanced production or clearance of endothelial proteins. Although few studies have focused on the mechanisms involved, Stratton et al[22] contend that an increase in endothelial protein production probably does not explain the enhanced fibrinolytic activity observed in physically trained individuals because they did not observe a posttraining increase in other endothelial proteins. It has also been hypothesized that changes in PAI-1 activity may be mediated by the lipid-related changes that occur with habitual exercise,[26] evidenced by the correlation that has been found between triglycerides and PAI.[27] Thus, many questions pertaining to the mechanisms involved in fibrinolytic changes with exercise are still unanswered; however, the present investigation was not designed to investigate potential mechanisms.

Impaired fibrinolysis, defined as either low TPA activity or elevated PAI-1 activity, has been associated with a variety of thromboembolic diseases.[28] Elevated PAI-1 activity has been documented in survivors of myocardial infarction[26,27] and individuals with coronary artery disease.[29-32] It is possible that the lower PAI-1 activity in physically active men compared with inactive men observed in the present study and by other investigators[26] is an important mechanism mediating the cardioprotective effect afforded by participation in regular physical activity.[33] Since thrombosis appears to be the immediate cause of most myocardial infarctions,[34] impaired fibrinolytic activity may play a crucial role in its pathology. Participation in physical activity may be a relatively simple yet meaningful intervention to beneficially alter the fibrinolytic system.

Although moderate exercise produced significant increases in TPA activity for both groups during both morning and evening, there was a significant interaction between time of day of exercise performance and time, with evening exercise producing greater increases in TPA activity than morning exercise. The evening exercise session in the active group elicited the greatest response. The mechanism responsible for the greater increases during the evening is not completely understood, although it is most likely due to the underlying diurnal variations at rest.[7-11] PAI-1 is hypothesized to be the major regulator of diurnal variations. Researchers have found that although TPA activity is lowest in the morning, TPA antigen is at its highest.[7,12] This suggests that PAI-1 does not affect TPA production and/or release but rather forms an inactive complex with TPA, subsequently lowering the amount of active TPA.[7,12] This may also be the case during exercise. The higher TPA activity observed during evening exercise may in part be explained by the lower PAI-1 activity, resulting in fewer TPA–PAI-1 complexes and thus more active TPA.

Whether this enhanced fibrinolytic activity during the evening is physiologically important is unknown. Unfortunately, it has been suggested, particularly in the lay literature, that morning exercise may be more likely to precipitate a cardiac event than evening exercise. This potentially misleading hypothesis is based on a number of points, including (1) the knowledge that the incidence of serious cardiac events is higher during the morning hours,[35,36] (2) the finding that exercise enhances coagulation,[3,37] and (3) the well-known diurnal variations in fibrinolytic activity, which is lowest during the morning. However, no data lend credibility to this allegation. On the contrary, a recent report[38] designed to examine the safety of morning versus afternoon exercise in cardiac patients refutes this hypothesis. In fact, the incidence of severe cardiac events during submaximal exercise was very low in this high-risk group, regardless of whether the exercise was performed in the morning or afternoon. No statistically significant difference in incidence rates was observed, with 3.0 cardiac events per 100 000 patient-hours in the morning and 2.4 in the afternoon. Thus, although exercise enhances coagulation and fibrinolytic activity is lower in the morning, most available data suggest that the coagulation and fibrinolytic systems remain in balance in response to stress.[37] Furthermore, the general consensus is that the overall risk of experiencing a cardiovascular event with exercise at any time of day is transient and very low,[39] and the overall risk of dying is lower in physically fit individuals compared with their unfit counterparts.[40]

In summary, the major finding of this investigation is that moderate intensity exercise increases TPA activity in both physically active and inactive men, with greater increases seen in active men, particularly during evening exercise. This may represent an increased ability to activate the fibrinolytic system in response to a stressor, such as potentially threatening microthrombi that may form in a coronary artery. These greater increases in TPA activity and the lower PAI-1 activity observed in the active men may be important factors mediating the

cardioprotective effect of regular physical activity. A well-controlled exercise training study is needed to provide more information on the role of the fibrinolytic system as a potential mechanism for the decreased incidence of coronary artery disease in physically active individuals and the amount of activity required to achieve beneficial adaptations.

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References

1. Andrew M, Carter C, O'Brodovich H, Heigenhauser G. Increases in factor VIII complex and fibrinolytic activity are dependent on exercise intensity. *J Appl Physiol.* 1986;60:1917-1922.
2. Davis GL, Abildgaard CF, Bernauer EM, Britton M. Fibrinolytic and hemostatic changes during and after maximal exercise in males. *J Appl Physiol.* 1976;40:287-292.
3. Drygas WK. Changes in blood platelet function, coagulation, and fibrinolytic activity in response to moderate, exhaustive, and prolonged exercise. *Int J Sports Med.* 1988;9:67-72.
4. El-Sayed MS. Exercise intensity-related responses of fibrinolytic activity and vasopressin in man. *Med Sci Sports Exerc.* 1990;22: 494-500.
5. 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 Physiol.* 1987;62:1416-1421.
6. Szymanski LM, Pate RR, Durstine JL. Effects of maximal exercise and venous occlusion on fibrinolytic activity in physically active and inactive men. *J Appl Physiol.* In press.
7. Angleton P, Chandler WL, Schmer G. Diurnal variation of tissue-type plasminogen activator and its rapid inhibitor (PAI-1). *Circulation.* 1989;79:101-106.
8. Grimaudo V, Hauert J, Bachmann F, Kruithof KO. Diurnal variation of the fibrinolytic system. *Thromb Haemost.* 1988;59: 495-499.
9. Kluft C, Jie AFH, Rijken DC, Verheijen JH. Daytime fluctuations in blood of tissue-type plasminogen activator (t-PA) and its fast-acting inhibitor (PAI-1). *Thromb Haemost.* 1988;59:329-332.
10. Rosing DR, Brakman P, Redwood DR, Goldstein RE, Beiser GD, Astrup T, Epstein SE. Blood fibrinolytic activity in man: diurnal variation and the response to varying intensities of exercise. *Circ Res.* 1970;27:171-184.
11. Takada A, Takada Y, Urano T, Sakakibara K, Rydzewski A. Fluctuations of euglobulin lysis time, tissue plasminogen activator, and free and total plasminogen activator inhibitor levels in plasma in daytime. *Thromb Res.* 1990;57:13-20.
12. Chandler WL, Trimble SL, Loo S-C, Mornin D. Effect of PAI-1 levels on the molar concentration of active tissue plasminogen activator (t-PA) and t-PA/PAI-1 complex in plasma. *Blood.* 1990; 76:930-937.
13. Szymanski LM, Pate RR, Durstine JL. Effects of exercise intensity, duration and time of day on fibrinolytic activity in physically active men. *Med Sci Sports Exerc.* 1994;26:1102-1108.
14. Balke B, Ware RW. An experimental study of physical fitness of Air Force personnel. *US Armed Forces Med J.* 1959;10:675-688.
15. Drabkin OL, Austin JH. Spectrophotometric studies, II: preparation from washed cells; nitric oxide, hemoglobin, and sulfhemoglobin. *J Biol Chem.* 1935;112:51-55.
16. Dill D, Costill D. Calculation of percentage changes in volumes of blood, plasma and red cells in dehydration. *J Appl Physiol.* 1974; 37:247-248.
17. Chandler WL, Schmer G, Stratton JR. Optimum conditions for the stabilization and measurement of tissue plasminogen activator activity in human plasma. *J Lab Clin Med.* 1989;113:362-371.
18. Chandler WL, Loo SC, Nguyen SV, Schmer G, Stratton JR. Standardization of methods for measuring plasminogen activator inhibitor activity in human plasma. *Clin Chem.* 1989;35:787-793.
19. Jackson AS, Pollock ML. Generalized equations for predicting body density of man. *Br J Nutr.* 1978;40:497-504.
20. Menon S, Burke F, Dewar HA. Effect of strenuous and graded exercise on fibrinolytic activity. *Lancet.* 1967;1:700-702.
21. Gris JC, Schved JF, Aguilar-Martinez P, Sanchez N. Impact of physical training on plasminogen activator inhibitor activity in sedentary men. *Fibrinolysis.* 1990;4(suppl 2):97-98.
22. Stratton JR, Chandler WL, Schwartz RS, Cerqueira MD, Levy WC, Kahn SE, Larson VG, Cain KC, Beard JC, Abrass IB. Effects of physical conditioning on fibrinolytic variables and fibrinogen in young and old healthy adults. *Circulation.* 1991;83:1692-1697.
23. Chandler WL, Levy WC, Veith RC, Stratton JR. A kinetic model of the circulatory regulation of tissue plasminogen activator during exercise, epinephrine infusion, and endurance training. *Blood.* 1993;81:3293-3302.
24. Chandler WL, Veith RC, Fellingham GW, Levy WC, Schwartz RS, Cerqueira MD, Kahn SE, Larson VG, Cain KC, Beart JC, et al. Fibrinolytic response during exercise and epinephrine infusion in the same subjects. *J Am Coll Cardiol.* 1992;19:1412-1420.
25. deBoer A, Kluft C, Kroon JM, Kasper FJ, Schoemaker HC, Pruis J, Breimer DD, Soons PA, Emeis JJ, Cohen AF. Liver blood flow as a major determinant of the clearance of recombinant human tissue-type plasminogen activator. *Thromb Haemost.* 1992;67: 83-87.
26. Speiser W, Langer W, Pschaick A, Selmayr E, Ibe B, Nowacki PE, Muller-Berghaus G. Increased blood fibrinolytic activity after physical exercise: comparative study in individuals with different sporting activities and in patients after myocardial infarction taking part in a rehabilitation sports program. *Thromb Res.* 1988;51: 543-555.
27. Hamsten A, Wiman B, DeFaire U, Blombäck M. Increased plasma levels of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. *N Engl J Med.* 1985;313: 1557-1563.
28. Nilsson IM, Ljungner H, Tengborn L. Two different mechanisms in patients with venous thrombosis and defective fibrinolysis: Low concentration of plasminogen activator or increased concentration of plasminogen activator inhibitor. *Br Med J.* 1985;290:1453-1456.
29. Almer L-O, Ohlin H. Elevated levels of the rapid inhibitor of plasminogen activator (t-PAI) in acute myocardial infarction. *Thromb Res.* 1987;47:335-339.
30. Aznar J, Estelles A, Tormo G, Sapena P, Tormo V, Blanch S, España F. Plasminogen activator inhibitor activity and other fibrinolytic variables in patients with coronary artery disease. *Br Heart J.* 1988;59:535-541.
31. Hamsten A, DeFaire U, Walldius G, Dahlen G, Szamosi A, Landou C, Blomback M, Wiman B. Plasminogen activator inhibitor in plasma: risk factor for recurrent myocardial infarction. *Lancet.* 1987;2:3-9.
32. Paramo JA, Colucci M, Collen D, Van de Werf F. Plasminogen activator inhibitor in the blood of patients with coronary artery disease. *Br Med J.* 1985;291:573-574.
33. Powell KE, Thompson PD, Casperson CJ, Kendrick JS. Physical activity and the incidence of coronary heart disease. *Annu Rev Public Health.* 1987;8:253-287.
34. DeWood MA, Spores J, Notske R, Mouser LT, Burroughs R, Golden MS, Lang HT. Prevalence of total coronary occlusion during the early hours of transmural myocardial infarction. *N Engl J Med.* 1980;303:897-902.
35. Muller JE, Stone PH, Turi ZG, Rutherford JD, Czeisler CA, Parker C, Poole WK, Passamani E, Roberts R, Robertson T, et al and the MILIS Study Group. Circadian variation in the frequency of onset of acute myocardial infarction. *N Engl J Med.* 1985;313: 1315-1322.
36. Muller JE, Ludmer PL, Willich SN, Tofler GH, Aylmer G, Klangos I, Stone PH. Circadian variation in the frequency of sudden cardiac death. *Circulation.* 1987;75:131-138.
37. Winther K, Hillegass W, Tofler GH, Jimenez A, Brezinski DA, Schafer AI, Loscalzo J, Williams GH, Muller JE. Effects on platelet aggregation and fibrinolytic activity during upright posture and exercise in healthy men. *Am J Cardiol.* 1992;70:1051-1055.
38. Murray PM, Herrington DM, Pettus CW, Miller HS, Cantwell JD, Little WC. Should patients with heart disease exercise in the morning or afternoon? *Arch Intern Med.* 1993;153:833-836.
39. Siscovick DS, Weiss NS, Fletcher RH, Lasky T. The incidence of primary cardiac arrest during vigorous exercise. *N Engl J Med.* 1984;311:874-877.
40. Blair SN, Kohl HW, Paffenbarger RS Jr, Clark DG, Cooper KH, Gibbons LW. Physical fitness and all-cause mortality: a prospective study of healthy men and women. *JAMA.* 1989;262:2395-2401.