Fibrinolytic responses to acute physical activity in older hypertensive men

CHRISTOPHER A. DESOUZA,1 DONALD R. DENGEL,2 MARC A. ROGERS,1 KIM COX, AND RICHARD F. MACKO2,3

Department of Kinesiology, University of Maryland, College Park 20742; and Division of Gerontology, Department of Medicine, and Department of Neurology, University of Maryland Medical School and Baltimore Veterans Affairs Medical Center, Geriatric, Research, Education, and Clinical Center, Baltimore, Maryland 21201

DeSouza, Christopher A., Donald R. Dengel, Marc A. Rogers, Kim Cox, and Richard F. Macko. Fibrinolytic responses to acute physical activity in older hypertensive men. J. Appl. Physiol. 82(6): 1765–1770, 1997.—We tested the hypothesis that the fibrinolytic response to acute physical activity is impaired in sedentary older hypertensive men, which may contribute to the risk of exertion-triggered acute myocardial infarction in this population. Tissue-type plasminogen activator (t-PA) antigen and activity and plasminogen activator inhibitor-1 (PAI-1) antigen and activity were measured in 12 hypertensive (69 ± 1 yr) and 11 normotensive (64 ± 1 yr) men before and after an acute bout of submaximal exercise. Contrary to our hypothesis, there were no differences between the two groups in the fibrinolytic response to exercise. t-PA antigen and activity were significantly elevated in both the hypertensive (38 and 172%, respectively) and normotensive (45 and 130%, respectively) groups immediately after exercise but they returned to resting levels within 30 min. There was no change in PAI-1 antigen levels immediately after exercise in either group; however, PAI-1 antigen was significantly lower at 30 and 60 min postexercise in both the hypertensive (31 and 16%, respectively) and normotensive (35 and 20%, respectively) groups. PAI-1 activity was significantly lower immediately after exercise in both the hypertensive (25%) and normotensive (22%) groups and remained lower than preexercise levels at 30 min (23 and 26%, respectively) and 60 min (16 and 12%, respectively) postexercise in both groups. The results of this study demonstrate that the fibrinolytic response to an acute bout of moderate physical activity is not impaired in sedentary older hypertensive men.

fibrinolysis; exercise; tissue-type plasminogen activator; plasminogen activator inhibitor-1

METHODS

Subjects. Twelve hypertensive [69 ± 1 (SE) yr] and 11 healthy normotensive (65 ± 1 yr) sedentary male subjects volunteered to participate in this study. All subjects were free of overt cardiovascular, liver, endocrine-metabolic, and hematologic disease, as assessed by medical history and physical examination, fasting blood chemistries, a resting 12-lead electrocardiogram, and an oral glucose-tolerance test. Two hypertensive subjects who were being treated with antihypertensive medications were gradually tapered off their medication and studied after a minimum of 4 wk of no-drug therapy. All subjects were nonsmokers, free of prescription or over-the-counter medication that may affect blood coagulation, and had not participated in a regular aerobic exercise program for at least 6 mo before the start of the study. The subjects provided written informed consent according to the guidelines of both the University of Maryland at Baltimore and

APPROXIMATELY 1.5 MILLION myocardial infarctions occur annually in the United States, resulting in close to 500,000 deaths. At least 75,000 of these infarctions are estimated to occur during or within 1 h after physical exertion (17). Recent epidemiological data have shown that physical exertion/activity may trigger the onset of acute myocardial infarction, particularly in sedentary older adults who are at increased cardiovascular risk (17, 28, 29). One proposed mechanism for this phenomenon is the disruption of a vulnerable, but not occlusive, atherosclerotic plaque due to increased hemodynamic shear stresses (17, 20). The exposed atherogenic surface is then exposed to hemostatic regulatory processes that modulate thrombus deposition and dissolution (11, 18).
University of Maryland at College Park Institutional Review Boards.

Measurement of blood pressure. Before having their blood pressure measured, the subjects rested quietly in a seated position for 15 min. Blood pressure was measured by using an automated Dinamap (Critikon, 1486SX, Tampa, FL) blood-pressure monitor with the appropriate size cuff. Triplicate measurements were made 2 min apart and averaged on 3 separate days over a period of 2 wk. Hypertension was defined as mean systolic blood pressure of 140 mmHg or greater and/or mean diastolic blood pressure of 90 mmHg or greater (14).

Measurement of body composition. Body weight was measured to the nearest 0.1 kg by using a medical beam balance (Detecto, Webb City, MO), and height was measured to the nearest 0.5 cm. Percent body fat was determined by dual-energy X-ray absorptiometry (Lunar Radiation Corporation, Madison, WI), and fat-free mass was calculated as kilograms of body weight minus kilograms of fat mass. The waist-to-hip ratio was calculated as the ratio of the minimal waist circumference to the circumference of the maximal gluteal protuberance. Body mass index was calculated as weight (kg) divided by height squared (m²).

Measurement of maximal oxygen consumption (V\text{O}_2\text{max}). The subjects performed a maximal graded exercise test on a treadmill to screen for previously undiagnosed cardiovascular disease and to determine V\text{O}_2\text{max} by using the Bruce protocol (3). Heart rate, blood pressure, and a 12-lead electrocardiogram were recorded at the end of each stage. Inspired air volume was measured by using a Rayfield equipment gas meter (Waitsfield, VT). Concentrations of expired oxygen and carbon dioxide were analyzed from a mixing chamber with the use of Ametek 5-3A/1 and CD 3A analyzers, respectively. Standard gases were used to calibrate both analyzers before each test. Ventilation, oxygen consumption (V\text{O}_2), carbon dioxide production, and respiratory exchange ratio (RER) were measured continuously during each test by a computerized data-acquisition system interfaced with the gas meter and gas analyzers. A true V\text{O}_2\text{max} was accepted when at least two of the following criteria were met: 1) a plateau in V\text{O}_2 with increasing work rate (<2 l/min or <200 ml kg⁻¹ min⁻¹); 2) RER at maximal exercise >1.10 and; 3) maximal heart rate >95% of age-predicted maximum (220 – age). Subjects who showed no evidence of cardiovascular decompensation during the maximal graded exercise test and were determined to have a negative test by the attending physician were allowed to participate in the study.

Acute submaximal exercise test. The subjects performed an acute submaximal bout of aerobic exercise that consisted of walking on a treadmill for 30 min at 65% of V\text{O}_2\text{max}. This mode and intensity of physical activity was chosen because it is recommended and generally prescribed in geriatric exercise programs (1). To begin the 30-min bout of exercise, the subjects moved directly from a semirecumbent position to walking on the treadmill at 1 mile/h and 0% grade. The speed and grade were immediately increased to the estimated level that would elicit ~65% of the subject’s previously determined V\text{O}_2\text{max}. The appropriate speed and grade for each subject were estimated by using American College of Sports Medicine metabolic equations (1). To monitor relative exercise intensity, expired air was collected at 5, 15, and 25 min during exercise. If necessary, the speed and/or grade was adjusted accordingly to achieve and/or maintain the desired V\text{O}_2. Before each sampling period, both analyzers were calibrated with the use of standard gases. At the conclusion of the exercise test, the subjects immediately returned to the same semirecumbent position to facilitate all postexercise blood collections and to minimize the confounding effects of posture change on fibrinolytic variables.

Measurement of fibrinolytic variables. To avoid the known diurnal variation in fibrinolytic variables, all submaximal exercise tests began between 8:30 AM and 9:30 AM after a 12-h overnight fast. Plasma levels of t-PA and PAI-1 antigen and activity were measured immediately before and after exercise and at 30 and 60 min postexercise. Phlebotomy without tourniquet was performed by using a 20-gauge polyethylene intravenous catheter. A 15-cm intravenous extension was attached to the end of the catheter to facilitate multiple blood sampling. Both the catheter and extension were kept patent throughout with a slow infusion of saline (0.9% saline solution). After catheterization, the subjects rested in a semirecumbent position for 20 min before the preexercise blood sample was collected. During phlebotomy, the first 2–3 ml of blood were discarded and samples were used only if venous return was prompt throughout. Blood for the determination of t-PA antigen and t-PA activity was collected in a 10-ml syringe containing 1.0 ml of 130 mmol/l sodium citrate (final dilution volume 1:10). To prevent in vitro inactivation of t-PA by ongoing complex formation with PAI-1, 0.75 ml of citrate-anticoagulated whole blood was acidified within 1 min of phlebotomy by addition of 0.37 ml of 0.5 mmol/l sodium acetate, pH 4.2 (4). Blood samples to measure PAI-1 antigen and PAI-1 activity were collected in a 5-ml syringe containing modified Files solution (1 ml acid citrate dextrose solution, 80 µl of acetyl salicylic acid solution, and 10 µl prostaglandin E₁ solution) (10) to minimize in vitro platelet activation (final dilution volume 1:5). Within 30 min of phlebotomy, all samples were centrifuged for 20 min at 6,000 g at 4°C. Platelet-poor plasma was aliquoted and stored at −80°C until assayed at the end of the study. All t-PA and PAI-1 assays were performed in duplicate, with a maximum of one freeze-thaw cycle. Intra-assay variability was calculated from duplicate samples, and internal controls were used to determine interassay variability for all fibrinolytic assays. t-PA antigen and PAI-1 antigen were determined by using an enzyme-linked immunosorbent assay (American Bioproducts, Parsippany, NJ). t-PA activity and PAI-1 activity were measured by using an amidolytic method (Chromogenix, Franklin, OH). t-PA activity by phlebotomy by addition of 0.37 ml of 0.5 mmol/l sodium acetate, pH 4.2 (4). Blood samples to measure PAI-1 antigen and PAI-1 activity were collected in a 5-ml syringe containing modified Files solution (1 ml acid citrate dextrose solution, 80 µl of acetyl salicylic acid solution, and 10 µl prostaglandin E₁ solution) (10) to minimize in vitro platelet activation (final dilution volume 1:5). Within 30 min of phlebotomy, all samples were centrifuged for 20 min at 6,000 g at 4°C. Platelet-poor plasma was aliquoted and stored at −80°C until assayed at the end of the study. All t-PA and PAI-1 assays were performed in duplicate, with a maximum of one freeze-thaw cycle. Intra-assay variability was calculated from duplicate samples, and internal controls were used to determine interassay variability for all fibrinolytic assays. t-PA activity and PAI-1 activity were determined by using an enzyme-linked immunosorbent assay (American Bioproducts, Parsippany, NJ). t-PA activity and PAI-1 activity were measured by using an amidolytic method (Chromogenix, Franklin, OH). t-PA activity and PAI-1 activity were measured by using an amidolytic method (Chromogenix, Franklin, OH). t-PA activity and PAI-1 activity were measured by using an amidolytic method (Chromogenix, Franklin, OH).

Statistical analysis. Repeated-measures analysis of variance was performed to determine statistical significance
between the hypertensive and normotensive groups. Planned mean comparisons were conducted to test for the effect of time on each dependent variable. Differences between the two groups for selected variables were tested by using a unpaired Student’s t-test. The level of statistical significance was set at P < 0.05. All data are presented as means ± SE.

RESULTS

Physical characteristics of the subjects. Baseline anthropometric and hemodynamic characteristics of the hypertensive and normotensive groups are presented in Table 1. There were no significant differences in body weight, percent body fat, fat-free mass, waist-to-hip ratio, or body mass index between the two groups. In accordance with the enrollment criteria, the hypertensive subjects had significantly higher systolic, diastolic, and mean arterial blood pressures than their normotensive peers.

Physiological responses to maximal and submaximal exercise. The hypertensive and normotensive subjects did not differ significantly in maximal or submaximal VO₂, whether expressed in absolute terms (l/min) or relative to body mass (ml·kg⁻¹·min⁻¹) (Table 2). The maximal and submaximal heart rates and RER achieved during the respective tests were also similar between the two groups. Both the hypertensive and normotensive groups performed the 30-min bout of submaximal aerobic exercise, at the same relative intensity (65 ± 2 and 64 ± 2% of VO₂max, respectively).

Fibrinolytic responses to submaximal aerobic exercise. There were no significant differences between the hypertensive and normotensive groups in the time course and magnitude of change in either t-PA antigen or t-PA activity in response to the 30-min bout of submaximal exercise. t-PA antigen increased by 38% (from 7.3 ± 0.5 to 10.1 ± 0.9 ng/ml), and t-PA activity increased by 172% (from 1.8 ± 0.3 to 4.9 ± 0.9 U/ml) in the hypertensive group, whereas in the normotensive group t-PA antigen and t-PA activity increased by 45% (from 6.2 ± 0.6 to 9.0 ± 0.9 ng/ml) and by 130% (from 1.7 ± 0.2 to 3.9 ± 0.6 U/ml), respectively. However, 30 min after cessation of exercise, both t-PA antigen and t-PA activity had returned to preexercise levels and were unchanged at 60 min postexercise in both groups (Fig. 1).

There were no changes in PAI-1 antigen levels immediately after exercise in either the hypertensive (from 14.2 ± 2.3 to 12.5 ± 2.5 ng/ml) or normotensive (from

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Table 1. Body weight, body composition, and blood pressure of the hypertensive and normotensive groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hypertensive (n = 12)</th>
<th>Normotensive (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>93.1 ± 2.6</td>
<td>93.2 ± 3.5</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>31.4 ± 1.6</td>
<td>28.6 ± 1.5</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>63.5 ± 1.0</td>
<td>66.4 ± 2.6</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>1.00 ± 0.22</td>
<td>0.97 ± 0.24</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>30.7 ± 0.8</td>
<td>29.5 ± 0.9</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td>153 ± 2*</td>
<td>120 ± 3</td>
</tr>
<tr>
<td>Systolic</td>
<td>81 ± 1*</td>
<td>71 ± 2</td>
</tr>
<tr>
<td>Diastolic</td>
<td>105 ± 1*</td>
<td>88 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. Mean arterial pressure was calculated as two-thirds diastolic blood pressure plus one-third systolic blood pressure. *Significantly different from normotensive group, P < 0.01.

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Table 2. Physiological responses to maximal and submaximal aerobic exercise in the hypertensive and normotensive groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hypertensive (n = 12)</th>
<th>Normotensive (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V̇O₂max, l/min</td>
<td>2.18 ± 0.08</td>
<td>2.34 ± 0.15</td>
</tr>
<tr>
<td>V̇O₂max, ml·kg⁻¹·min⁻¹</td>
<td>23.6 ± 1.0</td>
<td>24.9 ± 1.5</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>157 ± 3</td>
<td>154 ± 4</td>
</tr>
<tr>
<td>Respiratory exchange ratio</td>
<td>1.22 ± 0.02</td>
<td>1.15 ± 0.02</td>
</tr>
<tr>
<td>Maximal exercise</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V̇O₂, l/min</td>
<td>1.42 ± 0.05</td>
<td>1.48 ± 0.13</td>
</tr>
<tr>
<td>V̇O₂, ml·kg⁻¹·min⁻¹</td>
<td>15.4 ± 0.7</td>
<td>15.8 ± 1.2</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>107 ± 3</td>
<td>106 ± 4</td>
</tr>
<tr>
<td>Respiratory exchange ratio</td>
<td>0.92 ± 0.01</td>
<td>0.94 ± 0.02</td>
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</tbody>
</table>

Values are means ± SE; n, no. of subjects. V̇O₂, oxygen consumption; V̇O₂max, maximal V̇O₂.

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Fig. 1. Tissue-type plasminogen activator (t-PA) antigen (A) and t-PA activity (B) values immediately before and after exercise and at 30 and 60 min postexercise in hypertensive (●) and normotensive (○) groups. Values are means ± SE. †Significantly different from preexercise values, P < 0.01.
10.8 ± 2.2 to 11.4 ± 2.8 ng/ml) groups. However, at 30 and 60 min after the cessation of exercise, PAI-1 antigen levels were significantly lower than preexercise levels in both groups. In the hypertensive group, PAI-1 antigen decreased by 31% (9.8 ± 1.8 ng/ml) at 30 min and by 35% (9.1 ± 1.7 ng/ml) at 60 min postexercise, whereas in the normotensive group PAI-1 antigen decreased by 16% (9.1 ± 2.3 ng/ml) at 30 min and by 20% (8.6 ± 1.8 ng/ml) at 60 min postexercise (Fig. 2). Although the hypertensive subjects tended to have greater decreases in PAI-1 antigen levels at 30 and 60 min postexercise compared with the normotensive subjects, these differences were not significant.

In both the hypertensive and normotensive groups, PAI-1 activity was significantly lower immediately after exercise and remained lower than preexercise levels for up to 1 h after exercise. There was no difference in the magnitude of the decrease in PAI-1 activity after exercise between the two groups. In the hypertensive group, PAI-1 activity decreased by 25% (from 17.4 ± 1.2 to 13.0 ± 2.0 AU/ml) immediately after exercise and was 23% (13.3 ± 1.7 AU/ml) and 16% (14.6 ± 1.7 AU/ml) lower than preexercise levels at 30 and 60 min postexercise, respectively. Similarly, in the normotensive group, PAI-1 activity decreased by 22% (from 17.5 ± 1.8 to 13.7 ± 2.5 AU/ml) immediately after exercise and was 26% (12.9 ± 1.8 AU/ml) and 12% (15.5 ± 1.8 AU/ml) lower than preexercise levels at 30 and 60 min postexercise, respectively (Fig. 2).

DISCUSSION

The primary new finding from the present study was that sedentary older hypertensive men do not have an impaired fibrinolytic response to acute physical activity. The hypertensive subjects had similar increases in t-PA antigen and t-PA activity and concomitant decreases in PAI-1 antigen and PAI-1 activity in response to an acute bout of steady-state submaximal aerobic exercise compared with their normotensive peers. Although the increase in both t-PA antigen and t-PA activity was short lived in both groups, returning to preexercise levels within 30 min, the decrease in PAI-1 antigen and PAI-1 activity was sustained for up to 1 h after the cessation of exercise. To our knowledge, this is the first study to document the time course and magnitude of change in specific fibrinolytic variables following an acute bout of submaximal exercise in older adults. As such, the observed increase in t-PA activity and, specifically, the sustained decrease in PAI-1 activity after submaximal aerobic exercise, particularly in the hypertensive subjects who are at greater risk of thrombosis and sudden cardiac death (14, 19), may be of significant physiological importance.

Recently, three large epidemiological studies have reported that both moderate and heavy physical exertion/activity may trigger the onset of acute myocardial infarction, particularly in sedentary older individuals who are at increased cardiovascular risk (17, 28, 29). Although the precise mechanism(s) by which physical activity may trigger an acute myocardial infarction is unknown, the prevailing hypothesis involves plaque disruption and the activation of thrombogenic risk factors that favor clot formation vs. clot lysis (17, 29). These factors include platelet hyperactivity, increased fibrinogen concentration, and decreased fibrinolytic activity, due primarily to elevated PAI-1 activity (27, 29). Indeed, cadaver data have shown that in many cases the pathophysiology of an acute myocardial infarction involves the development of coronary thrombosis overlying a disrupted atherosclerotic plaque (9). In addition, it is well documented that increased PAI-1 activity is associated with, and contributes to, the development of acute atherothrombosis (15). PAI-1 is a highly regulated acute-phase protein that inhibits fibrinolytic activity by binding to and inactivating t-PA (15). Elevated PAI-1 levels have been reported in patients who have suffered multiple myocardial infarctions (16), restenosis after percutaneous transluminal coronary angioplasty (21), and reoclusion after a coronary aorta bypass (30). Moreover, increased PAI-1 gene expression, localization, and production have been observed in the intima of atherosclerotic human arteries (15). Thus, if PAI-1 levels are increased in the area of an atherosclerotic lesion, and, especially, if the PAI-1 is in the active form, an environment would be created that
would promote fibrin deposition and foster the development of a thrombus. However, the results of the present investigation do not support the involvement of a physical activity-induced hypofibrinolytic state. To the contrary, the increase in t-PA activity and particularly the sustained decrease in both PAI-1 antigen and PAI-1 activity for up to an hour after cessation of exercise may reduce the thrombotic risk associated with moderate physical activity by, at the very least, maintaining fibrinolytic capacity during the period of greatest risk. The magnitudes of increase in t-PA antigen and t-PA activity and decrease in PAI-1 activity in both the hypertensive and normotensive subjects immediately after exercise are similar to those previously reported in young healthy adults (24). For example, the exercise-induced increase in t-PA activity in our group of older men was comparable in magnitude to the increase (119%) reported by Szymanski and Pate (24) in young (age 35 yr) sedentary subjects after maximal exercise. In addition, it is important to note that regular physical activity also produces significant changes in fibrinolytic activity. Stratton and colleagues (23) demonstrated that chronic aerobic exercise can enhance fibrinolysis in older men by increasing resting levels of t-PA activity and decreasing PAI-1 activity. These hemostatic adaptations likely contribute to the cardioprotective effect of regular exercise (17, 29).

The results of the present study differ from the findings of Gleerup and co-workers (12), who reported no change in PAI-1 activity after submaximal exercise in a group of subjects with stage 1 hypertension. The reason for this discrepancy in findings is likely due to the difference in exercise stimulus employed in each study. Gleerup and colleagues (12) exercised their subjects for only 5 min at a moderate exercise intensity, compared with 30 min at 65% of VO2 max. In the present study, it is well established that the fibrinolytic response to acute aerobic exercise is dependent on both the intensity and duration of the exercise session (22, 25). Early work by Rosing et al. (22) eloquently showed that 5 min of exercise at VO2 max produced a significant increase in fibrinolytic activity, whereas 5 min of exercise at 70% and 40% of VO2 max produced only a small insignificant increase in fibrinolytic activity. However, after 30 min of exercise at 70% of VO2 max, fibrinolytic activity significantly increased to levels similar to those achieved after 5 min of near-maximal exercise. In contrast, although 30 min of exercise at 40% of VO2 max produced small but significant increases in fibrinolytic activity, the levels attained did not exceed the subjects peak diurnal levels. Thus it is likely that the exercise intensity employed by Gleerup and colleagues (12) was not sufficient enough to elicit a reduction in PAI-1 activity.

In conclusion, the results of the present study demonstrate that the fibrinolytic response to a typical bout of submaximal aerobic exercise is not impaired in sedentary older hypertensive men. Our findings suggest that an acute bout of moderate steady-state physical activity does not induce a hypofibrinolytic state that could contribute to the risk of an atherothrombotic event in older hypertensive men. It is important to note, however, that the exercise intensity employed in the present study (4.5 metabolic equivalents) was slightly lower than the exertion level identified by both Mittleman et al. (17) and Willich et al. (29) (6 metabolic equivalents) to be a potential trigger of an acute myocardial infarction. Thus we are unable to comment on the fibrinolytic response to higher intensity physical activity in older hypertensive adults. Finally, it is equally important to recognize that the fibrinolytic response to acute physical activity is only one potential hemostatic risk factor. Given the complexity of the coagulation and thrombo-lytic systems, future studies should continue to investigate the effect of acute physical activity on the thrombogenicity blood in different at-risk populations to gain further insight into the thrombotic mechanisms involved in physical activity-triggered acute vascular events.

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