Effects of Physical Conditioning on Fibrinolytic Variables and Fibrinogen in Young and Old Healthy Adults

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Background. The effects of 6 months of intensive endurance exercise training on resting tissue-type plasminogen activator (t-PA) activity, plasminogen activator inhibitor type 1 (PAI-1) activity, t-PA antigen, and fibrinogen were studied in 10 young (24–30 years) and in 13 old male subjects (60–82 years).

Methods and Results. After training, maximum oxygen consumption was increased in the young group by 18% (44.9±5.0 to 52.9±6.6 ml/kg/min, p<0.001), whereas it was increased in the old group by 22% (29.0±4.2 to 35.5±3.6 ml/kg/min, p<0.001). The young group had no significant changes in any of the measured variables, whereas the old group had a 39% increase in t-PA activity (0.82±0.47 to 1.14±0.42 IU/ml, p<0.03), a 141% increase in the percentage of t-PA in the active form (11.1±7.7 to 26.8±15.1%, p<0.01), a 58% decrease in PAI-1 activity (8.4±4.9 to 3.5±1.7 AU/ml, p<0.01), and a 13% decrease in fibrinogen (3.57±0.79 to 3.11±0.52 g/l, p<0.01).

Conclusions. We conclude that intensive exercise training enhances resting t-PA activity and reduces fibrinogen and PAI-1 activity in older men. These effects are potential mechanisms by which habitual physical activity might reduce the risk of cardiovascular disease. (Circulation 1991;83:1692–1697)

Habitual physical activity may reduce the risk of cardiovascular disease, but the mechanisms remain unclear.1–9 Intravascular thrombus formation is the final event leading to most acute vascular disorders. Acute thrombosis is a balance between the fibrinolytic mechanisms that favor thrombus breakdown and platelet, humoral, and vesel factors promoting thrombosis. Thus, the potential importance of fibrinolytic variables has become clearer, and several studies have suggested abnormalities of fibrinolysis in patients with coronary heart disease or myocardial infarction.10–19

Prior data regarding the effects of exercise training on fibrinolytic activity have been conflicting,20–27 which may in part be related to the relatively crude euglobulin lysis assays used in most studies. Fibrinolytic activity is largely regulated by tissue-type plasminogen activator (t-PA) activity and by a circulating inhibitor, plasminogen activator inhibitor type 1 (PAI-1). Recently, sensitive, specific, and reproducible assays for both t-PA activity and PAI-1 activity were developed and refined.28–30 Fibrinogen has emerged as an independent risk factor of importance equal to that of other previously described cardiovascular risk factors.31–34 Whether exercise (or other interventions) can reduce fibrinogen and, thereby, potentially decrease cardiovascular risk is unknown.

The purpose of this study was to determine whether intensive exercise training alters resting fibrinolytic variables and fibrinogen in healthy adult men. Because some prior studies suggested that fibrinolytic activity decreases with aging,10,35–37 we studied both a young and an old group.

Methods

Subjects

Twenty-three healthy male subjects who entered and completed an intensive 6-month endurance ex-
Assay

Training Program and Maximum \(\dot{V}_O_2\)

The 6-month endurance training program was intensive and closely supervised. Participants exercised four or five times per week beginning at 50–60% of heart rate reserve and increasing to 80–85% by the fourth month. The program consisted of walking, jogging, and bicycling for 45 minutes per session. Before and after 6 months of training, maximum \(\dot{V}_O_2\) was directly measured with a Bruce protocol maximal exercise (treadmill) test. The mean respiratory quotient was 1.23±0.09 on the pretests and 1.24±0.05\((p=\text{NS})\) on the post-tests, indicating excellent effort. A respiratory quotient of 1.2 or more was achieved on all but three of the 46 tests.

Assays

Because of the known diurnal variation in fibrinolytic variables, all samples were drawn between 8:00 and 11:15 AM after an overnight fast with the subject in a resting position for at least 20 minutes. The postexercise training samples were drawn at least 36 hours after the last episode of exercise to avoid the potential acute effects of exercise.

Methods for stabilizing and measuring t-PA activity and PAI-1 activity have been reported from our laboratory.\textsuperscript{28,30} t-PA activity was measured in acidified citrated plasma with an amidolytic method as previously described.\textsuperscript{29} Results were expressed in international units by comparison with a one-chain t-PA activity standard (NIBSC 86/670). PAI-1 activity was measured in plasma anticoagulated with 0.005 mol/l EGTA containing 0.005 mol/l reduced glutathione.\textsuperscript{30} There was no difference in PAI-1 activity measured in citrate or in EGTA anticoagulated samples. PAI-1 activity was determined by adding active one-chain t-PA (final concentration, 50 IU/ml) to an aliquot of plasma, followed by incubation for 15 minutes at 37°C. The concentration of t-PA/PAI-1 complex was measured in the plasma before and after addition of the excess t-PA by an enzyme-linked immunosorbent assay (ELISA) method as previously described.\textsuperscript{30} The concentration of active PAI-1 was equal to the difference in t-PA/PAI-1 complex before and after t-PA addition. Active PAI-1 levels in nanograms per milliliter were converted to activity units using a specific activity for PAI-1 of 0.89 arbitrary units (AU)/ng as previously determined.\textsuperscript{30} One arbitrary unit of PAI-1 was defined as the amount of PAI-1 that inhibits 1 IU of one-chain t-PA.

Total t-PA antigen was determined in citrated plasma with an ELISA,\textsuperscript{37,38} Polyclonal goat-anti-human t-PA and its peroxidase conjugate were obtained from American Diagnostica Inc. One-chain t-PA diluted in normal plasma was used to construct the standard curve. The percentage of total t-PA that was in the active form in vivo was estimated by use of the t-PA activity and total t-PA antigen measurements described above.\textsuperscript{30} First, the t-PA activity results were converted into molar concentrations by dividing the activity in international units per liter by the specific molar activity of t-PA: 4.48×10\textsuperscript{12} IU/mol. Next, the total t-PA antigen values were converted into molar concentrations by dividing the concentration in grams per liter by the molecular weight of t-PA: 65,000 g/mol.\textsuperscript{39} Last, the molar concentration of active t-PA was divided by the molar concentration of total t-PA, resulting in the fraction of circulating t-PA in the active form. Fibrinogen levels were measured by the method of Clauss.\textsuperscript{40}

Inflammatory and infectious disorders cause an acute phase response, which increases PAI-1 and fibrinogen. To exclude the presence of an acute phase response as a cause of changes in PAI-1 activity or fibrinogen, we also measured two other acute phase proteins, von Willebrand factor and C-reactive protein. Von Willebrand factor was measured by an ELISA method,\textsuperscript{41} and C-reactive protein was measured by a modified immunoturbidimetric method.\textsuperscript{42}

Statistical Analysis

Data are presented as the mean±SD in text and in the table and as the mean±SEM in the figures. Differences before and after exercise training were tested for significance by a two-tailed paired t test, and differences between the young and old groups before and after exercise training were tested for by an unpaired t test. A p value of less than 0.05 was considered significant.

Results

Both the old and young groups had significant increases in maximum \(\dot{V}_O_2\) (+22% and +18%, respectively, both \(p<0.001\)) (Table 1) and reductions in resting heart rates (−11 and −8 beats/min, respectively, both \(p<0.001\)). However, none of the fibrinolytic variables changed significantly in the young group (Table 1). The old group had a significant 39% increase in t-PA activity (from 0.82±0.47 to 1.14±0.42 IU/ml, \(p=0.03\)), whereas the young group had no change (from 0.67±0.31 to 0.65±0.37 IU/ml) (Figure 1). The 40% reduction in t-PA antigen was significant in the old group (from 12.6±5.4 to 7.7±3.1 ng/ml, \(p<0.01\)), but the 25% decrease in the young group (from 7.1±3.3 to 5.3±2.4 ng/ml) was not. The 141% increase in the percentage of total t-PA that was active was highly significant in the old group, but the 22% increase in the young group was not (Figure 2). PAI-1 decreased by 58% in the old group.
TABLE 1. Fibrinolytic Variables and Fibrinogen Before and After Exercise Training

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young (n=10)</th>
<th>Old (n=13)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td></td>
</tr>
<tr>
<td>(\dot{V}O_2)max (ml/kg/min)</td>
<td>44.9±5.0</td>
<td>52.9±6.6</td>
<td>0.001</td>
</tr>
<tr>
<td>t-PA activity (IU/ml)</td>
<td>0.67±0.31</td>
<td>0.65±0.37</td>
<td>NS</td>
</tr>
<tr>
<td>t-PA antigen (ng/ml)</td>
<td>7.1±3.3</td>
<td>5.3±2.4</td>
<td>NS</td>
</tr>
<tr>
<td>Percent active t-PA (%)</td>
<td>16.6±10.4</td>
<td>20.3±13.4</td>
<td>NS</td>
</tr>
<tr>
<td>PAI-1 activity (AU/ml)</td>
<td>4.2±3.3</td>
<td>3.6±2.0</td>
<td>NS</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>2.27±0.50</td>
<td>2.17±0.45</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td></td>
</tr>
<tr>
<td>(\dot{V}O_2)max (ml/kg/min)</td>
<td>29.0±4.2*</td>
<td>35.5±3.6*</td>
<td>0.001</td>
</tr>
<tr>
<td>t-PA activity (IU/ml)</td>
<td>0.82±0.47</td>
<td>1.14±0.42*</td>
<td>0.03</td>
</tr>
<tr>
<td>t-PA antigen (ng/ml)</td>
<td>12.6±5.4*</td>
<td>7.7±3.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Percent active t-PA (%)</td>
<td>11.1±7.7</td>
<td>26.8±15.1</td>
<td></td>
</tr>
<tr>
<td>PAI-1 activity (AU/ml)</td>
<td>8.4±4.9*</td>
<td>3.5±1.7</td>
<td>0.004</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>3.57±0.79*</td>
<td>3.11±0.52*</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05 young vs. old group.
Pre, before exercise training; Post, after exercise training; t-PA, tissue-type plasminogen activator; PAI-1, plasminogen activator inhibitor type 1.

(\(p<0.01\)) but by only 14% in the young group (\(p=\text{NS}\)) (Figure 3). Similarly, the 13% reduction in fibrinogen level was significant in the old group (from 3.57±0.79 to 3.11±0.52 g/l, \(p<0.01\)), but the 4% reduction in the young group was not (from 2.27±0.50 to 2.17±0.45 g/l).

At baseline, the old group had a significantly higher PAI-1 activity, t-PA antigen level, and fibrinogen level than the young group (all \(p<0.05\)), whereas t-PA activity and the percentage of t-PA in the active form were not significantly different. After training, the old group no longer had significantly higher values of PAI-1 and t-PA antigen than the young group, whereas fibrinogen level remained significantly higher. In addition, after training, the old group had a significantly higher t-PA activity level than did the young group, although the percentage of t-PA in the active form was not significantly different.

Values of von Willebrand factor before and after training were unchanged in both the old (1.10±0.28 and 1.06±0.31 units/ml, respectively) and young (0.77±0.27 and 0.71±0.21 units/ml, respectively) groups. Similarly, pretraining and post-training values for C-reactive protein were unchanged in the old (10.3±3.5 and 8.9±2.7 mg/l, respectively) and young (9.0±2.7 and 8.7±2.4 mg/l, respectively) groups.

Discussion

Several lines of evidence suggest that fibrinolytic abnormalities contribute to the risk of cardiovascular disease. A number of studies have detected higher levels of PAI-1 or lower levels of t-PA activity in subjects who had suffered a myocardial infarction compared with levels in controls.14,16–19,21 In addition, two prospective studies noted that reduced t-PA levels12 or increased PAI-1 levels16 were significant risk factors for reinfection. Whether t-PA or PAI-1 activity will emerge as independent predictors of cardiovascular events in subjects free of disease has not been determined.

In the Framingham study, the impact of fibrinogen as a separate risk factor for cardiovascular disease was of importance equal to that of other major risk factors, such as blood pressure, obesity, cigarette smoking, and diabetes.32 Although prior cross-sectional studies suggested that active, young healthy subjects had lower fibrinogen levels than sedentary subjects,21,25 our study is the first longitudinal study to demonstrate a reduction in fibrinogen levels by exercise training. Whether a reduction in fibrinogen level is associated with a reduction in cardiovascular events, as occurs with cholesterol lowering, has not yet been determined. Fibrinogen levels increase with age,52,33 and the old group had a mean fibrinogen level that was 1.16 g/l higher than the young group at baseline. With training, only the elderly group exhibited a significant decline in fibrinogen.

Epidemiological studies suggest that regular physical activity may reduce cardiovascular risk.1–9 Long-term exercise favorably modifies several of the conventional coronary heart disease risk factors including blood lipids, obesity, blood pressure, and insulin levels.1,43–45 However, the magnitude of change in these conventional risk factors is relatively moderate. For example, Thompson et al.44 with a vigorous training protocol that increased maximum \(\dot{V}O_2\) by

![t-PA Activity](image_url)
26%, found no change in low density lipoprotein cholesterol levels, a 17% decrease in triglyceride levels, and a 14% increase in high density lipoprotein cholesterol levels.

The present study suggests additional potential mechanisms for the possible favorable effect of exercise on cardiovascular risk. In the old group, mean t-PA increased by 39%, the percentage of t-PA circulating in the active form increased by 141%, PAI-1 decreased by 52%, and fibrinogen level decreased by 13%.

The fibrinolytic system antagonizes thrombosis by dissolving fibrin deposits. In the presence of fibrin, t-PA converts the proenzyme plasminogen into plasmin, which lysed the thrombus. Fibrinolytic activity in the blood is regulated by the rate of t-PA released from endothelial cells, the rapid destruction of t-PA by its inhibitor PAI-1 (a circulating protein produced by the endothelium and liver), and the rate of clearance of t-PA by the liver.46

Accurate measurements of specific components of the fibrinolytic system have been available only in the last several years. There have been 40- to 50-fold variations reported in the "normal" values of t-PA activity and PAI-1 activity, largely due to variations in collection and assay conditions. We recently reported optimized methods for the assay of both t-PA activity and PAI-1 activity,28–30 which were used in the present study.

The two principal forms of t-PA in the blood are active t-PA and inactive t-PA complexed to PAI-1 (t-PA/PAI-1 complex). Whereas the t-PA activity assay measures the amount of functioning, active t-PA that is present, the t-PA antigen assay measures t-PA present in any form. In most individuals, the majority of the t-PA in the blood is in the form of t-PA/PAI-1 complex,30 such that the percentage of active t-PA is low. After endurance training, the fraction of active t-PA increased, whereas the total level of t-PA fell. Fibrinolysis is initiated by the release of t-PA, primarily from endothelial cells. The lack of an increase in von Willebrand factor, which is also produced by endothelial cells, suggests that the increase in t-PA activity is not due to a generalized increase in protein production by the endothelial cell. This suggests that the concentration of t-PA/PAI-1 complex was decreasing. The cause of this decrease was not apparent. The findings are compatible with either reduced t-PA/PAI-1 complex or an increase in the clearance of t-PA/PAI-1 complex formation or both. A possible contributing factor that needs to be explored is the role of hepatic clearance. It has been suggested that old patients have decreased clearance of t-PA antigen compared with healthy young subjects. Endurance training may alter secretion and clearance parameters for different factors in the fibrinolytic system.

Most of both the old and young subjects demonstrated an increase in the fraction of active t-PA in the circulation. The change in the fraction of active t-PA was due to both an increase in the concentration of active t-PA and a decrease in total t-PA.

Most prior studies of the effects of exercise training on the fibrinolytic system have used relatively crude assays (largely euglobulin lysis assays) and offered conflicting results.20–27,47–49 In the longest longitudinal study, Williams et al22 found a reduction in resting fibrinolytic activity measured by an indirect euglobulin lysis assay but found an increase in fibrinolytic activity after venous occlusion. In addition, the potential benefit occurred only in women, not in men. The post venous occlusion test was recently criticized because of a poor specificity compared with other tests.50 In a cross-sectional study, Speiser et al21 found that young athletes had lower PAI-1 levels at rest than did young sedentary subjects; the active subjects also had a greater increase in t-PA activity during exercise than did the inactive subjects, but resting t-PA activity was not measured. In a recent longitudinal study, PAI-1 activity decreased in young men 25–29 years old who ran 5 kilometers every other day; t-PA levels, the percentage of active t-PA, and fibrinogen levels were not measured. Our longitudinal study also documents that long-term exercise

**Figure 2.** Plot of the percentage of tissue-type plasminogen activator (t-PA) in the active form after exercise training in the old and young groups. Exercise training significantly increased t-PA in the active form in the old group but not in the young group.

**Figure 3.** Plot of plasminogen activator inhibitor type 1 (PAI-1) activity after exercise training in the old and young groups. Exercise training significantly decreased PAI-1 activity in the old group but not in the young group.
significantly reduces PAI-1 activity in older men, whereas the slight decrease in the young group was not significant.

Despite similar training effects in the young and old groups, as measured by increases in maximum VO_2, the old group had significant changes in fibrinolytic variables, whereas the young did not. The explanation for this is unclear. Perhaps the study of larger numbers of subjects will result in significant findings in this group as well. Because of the marked increase in myocardial infarction, sudden death, stroke, and other diseases caused by arterial thrombosis in older men, our findings in the old group may be particularly important. Additional studies in larger numbers of subjects are needed to determine whether our results apply to younger populations.

Limitations of this study include the relatively small numbers of subjects in each of the groups, the lack of female subjects, and the lack of subjects with cardiovascular and other diseases in which the changes in fibrinolytic variables might be of greater importance. In addition, the 23-year age range of the old group was larger than the 7-year age range in the young group. The wider age range for the old group was necessitated by difficulties in recruiting rigorously screened healthy older men who were willing and able to undergo 6 months of intensive exercise training. Future studies should address the ability of exercise training to alter fibrinolytic variables in other groups and should also evaluate the effects of other training protocols, which would be more generally applicable than the highly intensive and supervised protocol used in this study.

We conclude that exercise training enhances fibrinolysis in healthy older men by increasing resting levels of t-PA activity and decreasing PAI-1 activity. Endurance training also reduces plasma fibrinogen in older men. These effects may be important mechanisms whereby regular physical activity reduces the risk of cardiovascular disease.

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