Association Between Physical Activity and Endogenous Fibrinolysis in Peripheral Arterial Disease: A Cross-sectional Study

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The purpose of this study was to determine whether daily physical activity was independently related to endogenous fibrinolysis in subjects with peripheral arterial disease (PAD). One hundred and six subjects with peripheral arterial disease (PAD) and intermittent claudication were characterized on the activity level of tissue plasminogen activator (tPA, the activator of fibrinolysis), the activity level of plasminogen activator inhibitor (PAI-1, the inhibitor of fibrinolysis), daily physical activity, ambulatory function, and demographic information. Subjects were separated into low (n = 36), moderate (n = 34), and high (n = 36) physical activity tertiles based on a 48-hour monitoring period with use of an accelerometer. The tPA activity of the low physical activity group (1.30 ±0.16 IU/mL) was 21% and 19% lower (p < 0.05) than that of the moderate (1.65 ±0.18 IU/mL) and the high (1.61 ±0.15 IU/mL) physical activity groups, respectively. The PAI-1 activity of the low physical activity group (21.41 ±1.14 AU/mL) was 15% and 23% higher than that of the moderate (18.61 ±1.34 AU/mL) and the high (17.47 ± 1.14 AU/mL) physical activity groups, respectively. Group differences in tPA activity and PAI-1 activity persisted after our controlling for group differences in measured and self-reported ambulatory measures. Daily physical activity is related to a more favorable endogenous fibrinolytic profile in PAD subjects with intermittent claudication. Subjects who expend fewer than 175 kcal/day in physical activities (approximately 35 minutes) are particularly susceptible to having a prothrombotic state. Subjects should be encouraged to participate in at least 35 minutes of physical activity each day to enhance fibrinolysis.

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**Introduction**

Formation of thrombus leads to progression of atherosclerotic disease and increases the risk of myocardial infarction and stroke. Peripheral arterial disease (PAD) subjects are particularly susceptible to these cardiovascular events owing to impairments in endogenous fibrinolysis favoring the formation of thrombus. The endogenous fibrinolytic system is regulated by the activity of tissue plasminogen activator (tPA), which lyses excess or inappropriately formed thrombus, and by tPA's inhibitor, plasminogen activator inhibitor-1 (PAI-1). Subjects with PAD have impaired fibrinolysis manifested by reduced tPA activity and elevated PAI-1 activity.

Fibrinolytic activity is improved through a program of exercise in healthy and coronary artery disease populations. However, no studies have examined whether this relationship exists in subjects with PAD limited by intermittent claudication. Therefore, the purpose of this study was to determine whether daily physical activity was independently related to endogenous fibrinolysis in subjects with PAD and intermittent claudication. We hypothesized that higher levels of daily physical activity would be associated with a more favorable endogenous fibrinolytic profile and that this relationship would be independent of ambulatory function and clinical characteristics.

**Methods**

**Subjects**

A total of 106 subjects with PAD were evaluated in the Geriatrics, Research, Education, and Clinical Center at the Maryland Veterans Affairs Health Care System (MVAHCS) at Baltimore. The subjects were recruited from the Vascular Clinic at the site of the Baltimore MVAHCS and from newspaper and radio advertisements soliciting those who experienced leg pain while they walked. Subjects underwent a medical history and physical examination. All subjects were classified as having Fontaine stage II PAD and were eligible to participate according to previously described inclusion and exclusion criteria. All subjects lived independently at home. The procedures used in this study were approved by the Institutional Review Boards at the University of Maryland and MVAHCS, Baltimore. Written informed consent was obtained from each patient before investigation.

**Measurements**

**Daily Physical Activity.** Physical activity level was monitored over 2 consecutive weekdays by a Caltrac accelerometer (Muscle Dynamics, Torrance, CA) attached to the belt of each subject as previously described. Subjects were grouped according to the low (n = 36), moderate (n = 34), and high (n = 36) tertiles of daily physical activity. The level of daily physical activity ranged between 17 and 175 kcal/day in the low tertile, between 176 and 350 kcal/day in the moderate tertile, and between 351 and 821 kcal/day in the high tertile.

**Endogenous Fibrinolysis.** Subjects underwent antecubital venipuncture without tourniquet-induced venostasis for quantification of fibrinolytic activity. Blood samples were collected at the same time of day to eliminate the known diurnal variations in fibrinolysis. Fibrinolysis was quantified by measurements of the biologic activities of tPA and PAI-1. For determination of tPA activity, blood was first collected into 130 mmol/L sodium citrate anticoagulant (9:1 volume) and immediately acidified by addition of 0.5 mmol/L sodium acetate, pH 4.2 (2:1 volume), to prevent the ongoing in vitro inactivation of tPA by complex formation with PAI-1. Samples for measurement of PAI-1 activity were collected into a 5 mL syringe containing modified Files solution (1 mL acid citrate dextrose solution, 80 µL acetylsalicylic acid solution, 10 µL prostaglandin E1) to minimize in vitro platelet activation (final dilution 1:5). Samples were maintained at 4°C until centrifugation at 10,000 x g for 20 minutes. Platelet-poor plasma was stored at −80°C until assays were performed.

The activity levels of tPA and PAI-1 were measured by use of an amidolytic method. All assays were performed in duplicate, and interassay variability was less than 5%. The tPA activity was expressed in international units (IU/mL) assessed against the 2nd International Standard for tPA from the National Institute for Biological Standards and Control. The PAI-1 activity was expressed in arbitrary units (AU/mL); 1 arbitrary unit of inhibitor is defined as the amount that inhibits 1 IU of tPA/mL plasma.

**Ankle/Brachial Pressure Index (ABPI).** After 10
minutes of supine rest, the ankle/brachial systolic blood pressures were obtained as previously described. The ABPI was calculated as ankle systolic pressure/brachial systolic pressure.

Treadmill Test. Subjects performed a progressive, graded treadmill protocol (2 mph, 0% grade with 2% increase every 2 minutes) until maximal claudication pain as previously described. The initial claudication distance (ICD), the absolute claudication distance (ACD), and peak oxygen uptake were measured.

Six-Minute Walk Test. Subjects performed an overground, 6-minute walk test supervised by trained exercise technicians as previously described. The distance to onset of claudication and the total distance walked during the test were measured.

Walking Impairment Questionnaire. Self-reported ambulatory ability was assessed by using a questionnaire validated for PAD subjects in which the subjects evaluate their walking ability at various speeds and distances, and their ability to climb stairs.

Statistical Analyses

One-factor analysis of variance (ANOVA) was per-

Table 1. Clinical characteristics of peripheral arterial disease (PAD) subjects separated into low, moderate, and high physical activity groups. Values are mean ± SEM and percentage of subjects in each category.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low Physical Activity Group (n = 35)</th>
<th>Moderate Physical Activity Group (n = 34)</th>
<th>High Physical Activity Group (n = 36)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>71 ± 1</td>
<td>70 ± 1</td>
<td>70 ± 1</td>
<td>0.749</td>
</tr>
<tr>
<td>ABPI</td>
<td>0.63 ± 0.02</td>
<td>0.63 ± 0.04</td>
<td>0.64 ± 0.02</td>
<td>0.651</td>
</tr>
<tr>
<td>Duration of IC (years)</td>
<td>7 ± 1</td>
<td>5 ± 1</td>
<td>6 ± 1</td>
<td>0.620</td>
</tr>
<tr>
<td>Walking distance to IC (blocks)</td>
<td>2.0 ± 0.3</td>
<td>2.3 ± 0.3</td>
<td>2.5 ± 0.3</td>
<td>0.243</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(% Caucasian)</td>
<td>53</td>
<td>56</td>
<td>61</td>
<td>0.212</td>
</tr>
<tr>
<td>(% African-American)</td>
<td>47</td>
<td>44</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Smoking history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(% Never)</td>
<td>6</td>
<td>9</td>
<td>8</td>
<td>0.923</td>
</tr>
<tr>
<td>(% Former)</td>
<td>61</td>
<td>47</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>(% Current)</td>
<td>33</td>
<td>44</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>33</td>
<td>41</td>
<td>25</td>
<td>0.419</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>84</td>
<td>65</td>
<td>50</td>
<td>0.297</td>
</tr>
<tr>
<td>Hyperlipidemia (%)</td>
<td>44</td>
<td>56</td>
<td>50</td>
<td>0.615</td>
</tr>
</tbody>
</table>

ABPI = ankle/brachial pressure index, IC = intermittent claudication.
formed to assess whether differences in tPA and PAI-1 existed among the 3 physical activity groups. Tukey post-hoc comparisons were performed to locate mean differences among the groups. One-factor ANOVAs, Tukey comparisons, and chi-square tests were used to assess whether differences in clinical characteristics and ambulatory function existed among the groups.

To assess whether group differences in tPA and PAI-1 were due to differences in clinical characteristics and ambulatory function among the 3 activity groups, stepwise multiple regression was subsequently performed to identify the independently related covariates of tPA and PAI-1. Analysis of covariance (ANCOVA) was then used to assess whether group differences in tPA and PAI-1 persisted after controlling for the significant covariates. The statistical assumptions of ANCOVA were tested and met for each covariate. All analyses were performed by use of the SPSS statistical package. Statistical significance was set at $p < 0.05$. Measurements are presented as means ± SEM.

### Results

#### Endogenous Fibrinolysis

The tPA activity of the low physical activity group (1.30 ±0.16 IU/mL) was 21% and 19% lower ($p < 0.05$) than that of the moderate (1.65 ±0.18 IU/mL) and the high (1.61 ±0.15 IU/mL) physical activity groups, respectively. The PAI-1 activity of the low physical activity group (21.41 ±1.14 AU/mL) was 15% and 23% higher than that of the moderate (18.61 ±1.34 AU/mL) and the high (17.47 ±1.14 AU/mL) physical activity groups, respectively.

#### Potential Covariates of Endogenous Fibrinolysis

The 3 physical activity groups were similar on all clinical characteristics (Table I). However, the measured and perceived ambulatory function was different among the groups (Table II). During the treadmill test, the low physical activity group had...
lower (p < 0.05) ICD, ACD, and peak oxygen uptake values than the moderate and high physical activity groups, and the moderate group had a shorter (p < 0.05) ACD than the high activity group. During the 6-minute walk test, the pain-free distance of the low physical activity group was shorter (p < 0.05) than that of the high activity group, and the total walking distance was different (p < 0.05) among all 3 groups. Perceived ambulatory function measured with the WIQ demonstrated that the high physical activity group had a greater (p < 0.05) walking speed score than either the low or moderate groups, and the high activity group had a greater (p < 0.05) stair climbing score than the low activity group. No group differences were noted for the WIQ distance score.

Adjusted Values of Endogenous Fibrinolysis

The variables that were significantly different among the physical activity groups in Table II were tested to determine if they were independently related (covariates) to the fibrinolytic measures. The speed component of the WIQ was the only significant (p < 0.05) covariate for tPA activity, and the 6-minute walk distance was the only significant (p < 0.05) covariate for PAI-1 activity. After our controlling for these covariates, group differences persisted for tPA activity and PAI-1 activity. The adjusted tPA activity of the low physical activity group (1.36 ±0.15 IU/mL) was 14% and 13% lower (p < 0.05) than that of the moderate (1.58 ±0.17 IU/mL) and the high (1.57 ±0.14 IU/mL) physical activity groups, respectively (Figure 1). The PAI-1 activity of the low physical activity group (21.12 ± 1.12 AU/mL) was 12% and 19% higher than that of the moderate (18.86 ±1.31 AU/mL) and the high (17.72 ± 1.11 AU/mL) physical activity groups, respectively (Figure 2).

Discussion

The major finding of this investigation was that subjects with the lowest levels of daily physical activity had impaired fibrinolysis compared to their more physically active counterparts. No dif-
ference in fibrinolysis existed between the moderate and high physical activity groups. Endogenous fibrinolytic dysfunction occurs through the mechanism of reduced tPA activity and increased PAI-1 activity, favoring a thrombotic state due to decreased fibrinolysis. These impairments in the fibrinolytic system increase the risk of cardiovascular events, such as myocardial infarction and stroke, and lead to a progression of atherosclerosis in the coronary arteries. Less is known about how the fibrinolytic system affects the peripheral arteries, although we have found that fibrinolysis is impaired in subjects with PAD and critical limb-threatening ischemia and intermittent claudication. Exercise training is an intervention that increases fibrinolysis in healthy and coronary artery disease populations. One study demonstrated that an acute bout of aerobic exercise improved fibrinolysis, manifested as increased tPA activity, in subjects with Fontaine stage II PAD. However, no studies have as yet addressed the effects of endurance exercise training on fibrinolytic activity in subjects with PAD.

The mechanism by which exercise increases fibrinolysis is unknown. Some data suggest that it may be related to weight loss and decreased fat content. Adipocytes produce large amounts of PAI-1, and in 1 study, weight loss was found to decrease PAI-1 in obese nondiabetic subjects. The notion that exercise promotes weight loss is well established. Elevations of PAI-1 are also related to particular genotypes, specifically the 4G/5G polymorphism, in the promoter of the PAI-1 gene. In another study, regular exercise decreased PAI-1 activity in subjects homozygous for the 4G allele, although not in subjects with other genotypes.

In the present study, exercise was quantified as the total daily physical activity directly measured by a validated accelerometer. PAD subjects who expended fewer than 175 kcal/day of physical activity were particularly susceptible to impaired fibrinolysis, manifested by a low tPA activity and a high PAI-1 activity. The PAD subjects who engaged in physical activity above this level had better fibrinolytic profiles, although they were still abnormal compared to age-matched controls. These results suggest that physical activity has a beneficial effect on fibrinolysis in subjects with PAD beyond a threshold level of 175 kcal/day but has little influence on activity levels ranging between 176 and 821 kcal/day. It remains possible that higher levels of physical activity are required to normalize tPA and PAI-1 activities in PAD subjects.

The clinical significance of this study is that subjects with PAD who are the least physically active and have the greatest ambulatory dysfunction can achieve the largest improvements in fibrinolysis by increasing their physical activity level. Since walking at a moderate pace burns approximately 5 kcal/min, and the physical activity threshold for enhanced fibrinolysis is 175 kcal/day, a recommendation that PAD subjects walk at least 35 min/day to lessen their risk of cardiovascular events appears warranted. This supports the recommendation by the Centers for Disease Control and the American College of Sports Medicine that every US adult should accumulate at least 30 minutes of physical activity per day to lessen cardiovascular risk. However, the cross-sectional design of this study is a limitation that needs to be recognized.

Although the exact mechanism by which exercise enhances fibrinolysis is not established, the numerous observations of the association between fibrinolysis and physical activity, as well as prospective exercise trials in many groups, establish this as an important method for decreasing cardiovascular morbidity and mortality. Until now, it has been unclear whether subjects with PAD, known to have impaired fibrinolysis but limited in their exercise capacity, could benefit from this method of fibrinolytic enhancement. This study is the first to establish a positive relationship between higher levels of physical activity and fibrinolytic enhancement in PAD subjects. A longitudinal design measuring the change in the fibrinolytic profile of PAD subjects during an exercise rehabilitation program is now needed to substantiate the beneficial effects of physical activity on tPA and PAI-1 activities in subjects with PAD limited by intermittent claudication.

Summary and Conclusion

The major finding of this investigation was that PAD subjects with the lowest levels of daily physical activity had impaired fibrinolysis compared to their more physically active counterparts. No difference in fibrinolysis existed between the moderate and high physical activity groups. In conclusion, daily physical activity is related to a more favorable endogenous fibrinolytic profile in PAD subjects with intermittent claudication. Subjects who expend fewer than 175 kcal/day in physical activities (approximately 35 minutes)
REFERENCES


