Relationship of physical fitness, hormone replacement therapy, and hemostatic risk factors in postmenopausal women

Linda M. Szymanski, Craig M. Kessler, and Bo Fernhall

1Department of OB/GYN, Johns Hopkins Medical Center, Baltimore, Maryland; 2Hematology Research Laboratory, Division of Hematology/Oncology, Georgetown University Medical Center, Washington, District of Columbia; and 3Kinesiology Department, College of Applied Life Studies, University of Illinois at Urbana-Champaign, Champaign, Illinois

Submitted 18 June 2004; accepted in final form 6 December 2004

Szymanski, Linda M., Craig M. Kessler, and Bo Fernhall. Relationship of physical fitness, hormone replacement therapy, and hemostatic risk factors in postmenopausal women. J Appl Physiol 98: 1341–1348, 2005. First published December 10, 2004; doi:10.1152/japplphysiol.00622.2004.—This cross-sectional study evaluated the relationship of physical fitness, hormone replacement therapy (HRT), and hemostatic profiles at rest and after an acute bout of maximal exercise in 48 healthy postmenopausal women. Subjects were categorized by fitness and HRT user status into four groups: unfit nonusers, fit nonusers, unfit users, and fit users. Fibrinolytic variables tissue plasminogen activator (tPA), plasminogen activator inhibitor-1 (PAI-1) activity, and antigen and prothrombin fragment 1 + 2, a molecular marker of in vivo thrombin generation, were measured before and after maximal exercise. Fibrinogen was also measured at rest. Higher tPA and lower PAI-1 activities (P < 0.05) were seen in HRT users and fit groups. tPA and PAI-1 antigens were lower in HRT and fit groups (P < 0.05) but not after correction for body mass index. Prothrombin fragment 1 + 2 was lower in the fit groups regardless of HRT status (P < 0.05). Fibrinogen was similar in all groups. Favorable hemostatic profiles were observed in physically fit compared with unfit women, especially in HRT nonusers. Thus fitness is more strongly related to these hemostatic risk factors compared with HRT since HRT did not affect these hemostatic variables in fit postmenopausal women.

fibrinolysis; coagulation; menopause; exercise; cardiovascular disease

CARDIOVASCULAR DISEASE (CVD) is the largest cause of death in women (2), and the risk of developing CVD increases after menopause (2, 15). Because thrombosis is thought to be a cause of most acute cardiovascular events (13), abnormalities in endogenous coagulation and fibrinolysis may play an important role in the risk of an acute cardiovascular event (20, 23, 25, 30). Aging is also associated with adverse changes in both coagulation and fibrinolysis (1, 11). For instance, postmenopausal women exhibit higher fibrinogen levels and lower levels of endogenous fibrinolysis [manifested as higher tissue plasminogen activator (tPA) antigen and lower plasminogen activator inhibitor-1 (PAI-1) activity and antigen] compared with premenopausal women (11), which may partially explain their higher risk of CVD.

Physical activity and physical fitness have consistently been linked to lower CVD rates in women (5, 28, 29). In fact, an inverse association between physical activity and total mortality (28), as well as a 50% reduction in risk of myocardial infarction (29), has been observed in physically active postmenopausal women. In addition to the numerous other benefits provided by regular physical activity, one of the mechanisms mediating the cardioprotective effect may be changes in the hemostatic system, particularly fibrinolysis (42, 45). Cross-sectional studies report greater fibrinolytic activity in physically active compared with inactive individuals (11, 45), including postmenopausal women. Longitudinal evidence also supports this notion (9, 38, 42). However, differences between physically fit and unfit individuals are not always present at rest but become exacerbated with a stressor, such as a maximal exercise test (44, 45). Thus maximal exercise testing can be used to better differentiate the fibrinolytic response between groups compared with using only resting levels of fibrinolysis (44, 45). Interestingly, physical fitness may have a more potent impact on endogenous fibrinolysis, particularly in response to maximal exercise, than other conditions known to adversely affect endogenous fibrinolysis, such as aging or CVD (11, 17). However, the fibrinolytic response to maximal exercise has not been investigated in postmenopausal women.

Hormone replacement therapy (HRT) has historically been promoted in postmenopausal women for CVD risk reduction because observational studies reported a cardioprotective effect (19, 39), possibly modulated by the improved hemostatic profiles (improvements in tPA antigen and PAI-1 activity) (18, 26, 37). However, HRT can also adversely affect hemostasis, and recent studies have questioned its cardioprotective effects (22, 35a). The prospective Heart Estrogen/progestin Replacement Study first reported a pattern of early increased risk of cardiac events and increased venous thromboembolic events with HRT (22). Subsequently, the Women’s Health Initiative showed a substantial increase in cardiovascular events in the HRT group compared with placebo after a 5.2-yr follow-up, but the risk was especially elevated during the first year of HRT therapy (35a). However, it should be recognized that HRT therapy is usually initiated due to postmenopausal symptoms, and HRT is very effective in eliminating such symptoms and increasing quality of life (43, 46). HRT also decreases the risk of bone fractures (4). Therefore, many women are likely to continue receiving HRT despite small increases in cardiovascular risks (43).

The increase in cardiovascular and thrombotic events associated with HRT is puzzling, especially considering the positive effects of HRT on fibrinolysis. Typically, HRT produces decreased levels of tPA antigen and PAI-1 activity and increased global fibrinolytic capacity (18, 26, 27, 37). These positive changes in fibrinolytic markers suggest there should be
a decreased not an increased risk of thromboembolic events with HRT. However, HRT also adversely affects the coagulation cascade. Increases in prothrombin fragment 1 + 2 (F1+2), a molecular marker of in vivo thrombin generation (27), that are elevated in persons at risk for CVD (31) could partially explain the increased risk of thromboembolic events. Furthermore, hormone supplements in young women (oral contraceptives) adversely affect the fibrinolytic response to exercise in addition to the increases in F1+2 (33). Taken together, these findings suggest that HRT may adversely affect the coagulation cascade and the fibrinolytic response to acute exercise. However, the interaction of acute exercise and HRT and the modulating effects of physical fitness on endogenous fibrinolysis are unknown in postmenopausal women. Thus investigating this interaction will provide an increased understanding of the effect of HRT on endogenous fibrinolysis in postmenopausal women and will elucidate whether the positive impact of physical fitness on fibrinolysis is modulated by HRT.

Therefore, the purpose of this study was to evaluate the fibrinolytic response to maximal exercise using a cross-sectional design comparing physically fit and unfit healthy postmenopausal women, both users and nonusers of HRT. The hemostatic risk factors measured included variables linked with CVD, including fibrinogen and the fibrinolytic variables tPA and PAI-1. In addition, F1+2 was included as a measure of coagulation activation. We hypothesized that physically fit women would exhibit greater increases in tPA activity and greater decreases in PAI-1 activity with acute exercise compared with unfit women, independent of body size. We also hypothesized that acute exercise would produce greater increases in tPA antigen in physically fit women but that PAI-1 antigen changes with exercise would not differ between fit and unfit women, also independent of body size. Furthermore, we hypothesized that unfit women on HRT would exhibit enhanced fibrinolytic profiles at rest but that the changes in tPA and PAI-1 activities and antigens would be reduced during acute exercise compared with unfit women not on HRT. Finally, we hypothesized that fit women on HRT would exhibit the best resting fibrinolytic profiles and the greatest changes in tPA and PAI-1 activities and antigens during acute exercise.

MATERIALS AND METHODS

Subjects. Forty-eight healthy postmenopausal women, both HRT users and nonusers, volunteered to participate. Eligible women were amenorrheic for >2 yr or aged >50 yr with no bleeding for at least 6 mo. Exclusion criteria included cigarette smoking, any prescribed medication other than HRT, including regular aspirin and nonsteroidal anti-inflammatory drug use, diagnosed hypertension, diabetes, hyperlipidemia, or personal history of CVD, thrombotic disorders, or thyroid disease. Only women with normal resting electrocardiograms and no evidence of exercise-induced ischemia or high-grade ventricular arrhythmias during a graded exercise test could participate. Women on HRT were eligible if they were taking combination estrogen-progestin therapy for at least 3 mo. Women taking cyclic progestosterone were studied when taking both estrogen and progestosterone. The University Medical Center Institutional Review Board approved the study, and written, informed consent was obtained from each subject before participation.

Design. Women were divided into four groups: physically unfit nonusers (No HRT-Unfit; n = 12), physically fit nonusers (No HRT-Fit; n = 12), physically unfit users (HRT-Unfit; n = 12), and physically fit users (HRT-Fit; n = 12). Hemostatic variables were measured at rest and after maximal exercise. All testing was conducted between 6:30 and 10:00 AM. Before being tested, subjects were instructed to fast (12 h), not engage in physical activity for at least 24 h, and not ingest aspirin or nonsteroidal anti-inflammatory drugs for 14 days.

Blood collection. Blood samples were collected by venipuncture from an antecubital vein with no or minimal stasis, 30 min after seated rest, and immediately after exercise using a 21-gauge needle. The first 3 ml of blood was discarded. Blood was then collected into tubes containing 50 µl of 15% K2-EDTA for hemoglobin and hematocrit determinations, 0.1 M sodium citrate for hemostasis determinations, and Biopool Stabilyte tubes containing 0.5 ml of 0.5 M citrate buffer, pH 4.3, for tPA activity stabilization. Blood was centrifuged at 2,600 g for 20 min. Plasma was separated, and aliquots were stored at −80°C.

Exercise test. All women underwent a physician-supervised maximal graded treadmill exercise test according to the Bruce protocol. Oxygen consumption was measured using an automated metabolic system (Quinton Q-Plex). Criteria for attaining maximal oxygen consumption included achieving two of the following: 1) plateau of oxygen consumption with increasing work rate (increase of <2.0 ml·kg⁻¹·min⁻¹), 2) respiratory exchange ratio of ≥1.05, and 3) maximum heart rate within 5 beats of age-predicted maximum. On the basis of the maximal oxygen consumption results, women were classified as fit (>29 ml·kg⁻¹·min⁻¹) or unfit (<29 ml·kg⁻¹·min⁻¹).

Blood analysis. Hemoglobin and hematocrit, measured using an automated cell counter (Baker Instruments; Hematology Series, system 9000), were used to correct postexercise results for plasma volume shifts (14). tPA and PAI-1 activities were determined using commercially available kits (Coasfit tPA, Coatest PAI; Chromogenix, Sweden), and antigens were measured using enzyme-linked immunoassay (Imubind Total tPA, Imubind Plasma PAI-1 ELISA; American Diagnostica, Greenwich, CT). F1+2 was measured using commercially available kits (Enzygnost F1+2 micro; Dade Behring, Germany). Fibrinogen was measured by the time titration method employing the ST-4 coagulation instrument (Diagnostica Stago). Only resting values of fibrinogen were analyzed. All samples from each subject were analyzed at the same time to control for potential interassay variations. The intra- and interassay coefficients of variation, respectively, for the hemostatic variables were as follows: tPA activity: 2.1 and 1.2%; tPA antigen: 7.1 and 2.3%; PAI-1 activity: 6.0 and 6.6%; PAI-1 antigen: 4.2 and 1.9%; F1+2: 4.8 and 7.7%; fibrinogen: 2.6 and 1.3%.

Other measures. Height and weight were measured on a standard scale. Body mass index (BMI) was calculated as weight (kg)/height (m²). Body fat percentage was assessed using the BOD POD body composition system (LIFE Measurement Instruments; Concord, CA). The waist-to-hip ratio (WHR) was calculated as the ratio of circumferences around the narrowest part of the torso and the maximal gluteal protuberance.

Statistical analysis. Two-way (HRT use × fitness status) ANOVA was used to compare descriptive variables among groups. To compare our data with most existing data, the first analysis included only resting levels of hemostatic variables using a two-way (HRT × fitness) ANOVA. Tukey’s post hoc multiple comparisons were used to probe significant differences. Then, to evaluate the effect of maximal exercise as a stressor of the hemostatic system, a three-way ANOVA with repeated measures (HRT status × fitness status × pre-/postexercise) was used. Selected predetermined comparisons were made using t-tests with Bonferroni’s correction for multiple comparisons to adjust the level of significance. Analysis of covariance statistically adjusted for differences in BMI between the fit and unfit subjects. Nondetectable levels of PAI-1 were entered as 0 for statistical analyses. Pearson correlation coefficients were calculated to assess associations between anthropometric, physical fitness, and hemostatic variables. The level of significance for all statistics was set at P < 0.05. All values are means ± SE, unless otherwise noted.
RESULTS

The physical characteristics and exercise test data are shown in Table 1. Groups were of similar age and years postmenopause. As expected, the physically fit women had significantly lower BMI, percent body fat, WHR, resting heart rate, and significantly higher maximum oxygen consumption and treadmill time than the unfit women, regardless of HRT status.

Fibrinolytic variables. tPA and PAI-1 activity results are shown in Fig. 1. Antigen results are shown in Fig. 2.

Resting tPA activity. Consistent with our hypotheses, women on HRT (P < 0.0001) and the fit women (P < 0.0001) exhibited higher resting tPA values, which were maintained when corrected for BMI (P < 0.0001 and P = 0.005, respectively). The No HRT-Unfit group exhibited lower resting, whereas the HRT-Fit group exhibited higher resting values than the other groups (P < 0.05), with no difference between the No HRT-Fit and HRT-Unfit groups.

Exercise tPA activity. There was a significant three-way (fitness × HRT × exercise; P = 0.008) interaction that was maintained after BMI was adjusted for. Consistent with our hypotheses, both groups of fit women exhibit greater increases in tPA activity with maximal exercise compared with the No HRT-Unfit group. In contrast to our hypothesis, the HRT-Unfit group also exhibited similar increases in tPA activities as the fit groups, and the HRT-Fit group was not different from the No HRT-Fit group.

Resting PAI-1 activity. Women on HRT (P = 0.003) and fit women (P < 0.0001) exhibited lower resting PAI-1 activity values, which remained significant even after correction for BMI (P = 0.007 and P = 0.024, respectively), consistent with our hypotheses. Also as we hypothesized, PAI-1 activity was higher in the No HRT-Unfit group compared with all other groups (P < 0.05). PAI-1 activity in the No HRT-Fit group was similar to both HRT groups.

Exercise PAI-1 activity. Significant main effects were seen for HRT (P = 0.002), fitness (P < 0.0001), and exercise (P < 0.0001), which were maintained after correcting for BMI (P = 0.007, P = 0.027, P = 0.027, respectively), consistent with our hypotheses. PAI-1 activity decreased with exercise in the No HRT-Fit and HRT-Unfit groups (P < 0.05). In contrast to our hypotheses, decreases with exercise in the HRT-Fit group did not reach significance (P > 0.05) (because of the Bonferroni correction). The No HRT-Unfit group did not change with exercise.

Resting tPA antigen. There was a significant main effect for fitness (P = 0.002) with lower levels in fit compared with unfit women, but this was not maintained when adjusted for BMI (P = 0.319). In contrast to our hypotheses, HRT did not significantly affect tPA antigen levels.

Exercise tPA antigen. When exercise data was evaluated, there was a significant main effect for exercise (P < 0.0001) that was maintained after adjusting for BMI (P = 0.016). There was also a fitness × exercise interaction (P = 0.001) showing fit women increased tPA antigen more than unfit women in response to exercise, consistent with our hypothesis. tPA antigen increased with acute exercise (P < 0.05) in all groups except the No HRT-Unfit group.

Resting PAI-1 antigen. Similar to tPA antigen results, when basal PAI-1 levels were analyzed, there was a significant main effect for fitness (P = 0.024) with lower PAI-1 antigen in fit vs. unfit groups, but this was not significant after adjusting for BMI.

Exercise PAI-1 antigen. In response to exercise, there were main effects for fitness (P = 0.018) and acute exercise (P = 0.008). Fit women had lower PAI-1 antigen, and there was a slight decrease in PAI-1 antigen with exercise. However, after correcting for BMI, none of these differences remained significant.

Coagulation. F1 + 2 results are shown in Fig. 3. At rest, there was a significant main effect for fitness (P = 0.006), with fit women exhibiting lower F1 + 2 levels than unfit women. These differences were maintained after adjusting for BMI (P = 0.033). HRT status approached significance (P = 0.07). There was no change in F1 + 2 with acute exercise (P > 0.05).

There were no differences in fibrinogen (P > 0.05) among the groups: No HRT-Unfit: 3.17 ± 0.24 g/l; No HRT-Fit: 2.95 ± 0.12 g/l; HRT-Unfit: 2.95 ± 0.17 g/l; HRT-Fit: 2.97 ± 0.16 g/l.

Correlations. Positive correlations between aerobic capacity and resting tPA activity (r = 0.47, P = 0.001) and negative correlations between aerobic capacity and basal tPA antigen (r = −0.49, P < .0001) and PAI-1 activity (r = −0.45, P = 0.002) were observed. BMI (r = −0.54, P < 0.0001), percent body fat (r = −0.40, P = 0.005), and WHR (r = −0.52, P < 0.0001) were all negatively correlated with tPA activity and

<table>
<thead>
<tr>
<th>Variables</th>
<th>No HRT</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Unfit</td>
<td>Fit</td>
</tr>
<tr>
<td>No. in group, n</td>
<td></td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Age, yr</td>
<td></td>
<td>60.6±8.6</td>
<td>55.9±4.9</td>
</tr>
<tr>
<td>Years postmenopause</td>
<td></td>
<td>9.1±6.3</td>
<td>6.6±5.6</td>
</tr>
<tr>
<td>Body mass index</td>
<td></td>
<td>28.8±4.7</td>
<td>22.5±2.0*</td>
</tr>
<tr>
<td>Body fat %</td>
<td></td>
<td>43.0±4.8</td>
<td>33.7±4.8*</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td></td>
<td>0.87±0.08</td>
<td>0.73±0.02*</td>
</tr>
<tr>
<td>Heart rate at rest, beats/min</td>
<td></td>
<td>80±11</td>
<td>67±7*</td>
</tr>
<tr>
<td>Maximum heart rate, beats/min</td>
<td></td>
<td>160±12</td>
<td>170±10</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td></td>
<td>135±16</td>
<td>128±21</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td></td>
<td>82±7</td>
<td>84±10</td>
</tr>
<tr>
<td>Maximal oxygen consumption, ml·kg⁻¹·min⁻¹</td>
<td></td>
<td>23.6±2.7</td>
<td>36.0±4.8*</td>
</tr>
<tr>
<td>Treadmill time, s</td>
<td></td>
<td>571±68</td>
<td>748±44*</td>
</tr>
<tr>
<td>Respiratory exchange ratio, maximal</td>
<td></td>
<td>1.13±0.07</td>
<td>1.26±0.07</td>
</tr>
</tbody>
</table>

Values are means ± SD. HRT, hormone replacement therapy. *Fit and unfit were significantly different (P < 0.05).
positively correlated with tPA antigen \((r = 0.51, P < 0.0001; r = 0.35, P = 0.017; r = 0.48, P = 0.001, \text{respectively})\) and PAI-1 activity \((r = 0.54, P < 0.0001; r = 0.42, P = 0.003; r = 0.64, P < 0.0001, \text{respectively})\). WHR and PAI-1 antigen were also correlated \((r = 0.41, P = 0.007)\). Correlations of BMI \((P = 0.091)\) and fitness \((P = 0.087)\) with fibrinogen were not significant.

**DISCUSSION**

There are several important new findings from this study. First, as hypothesized, the tPA activity response to maximal exercise was blunted in the unfit postmenopausal women. Although we hypothesized that the HRT-Fit group would show the greatest increase in tPA activity, fitness did not impact the response of tPA to maximal exercise in women on HRT. This finding suggests that fitness and HRT affected the tPA response to maximal exercise in a similar, but not additive, manner. Second, resting levels of tPA antigen were primarily affected by BMI, not by fitness or HRT, whereas the tPA antigen response to exercise was primarily affected by fitness but not by HRT. Thus fit postmenopausal women exhibited lower resting levels of tPA antigen (because of their lower BMI) but a higher tPA antigen response to exercise (not BMI dependent), independent of HRT status. Third, both fitness and HRT were associated with lower resting PAI-1 activity independently of BMI, and the effects were not additive. The response of PAI-1 activity to exercise was affected by both fitness and HRT, and unfit women not on HRT showed an unfavorable exercise response. Fourth, for PAI-1 antigen, both resting levels and the response to exercise were independent of both fitness and HRT after accounting for BMI. Last, fitness, but not HRT, impacted resting coagulation activation, but there were no associations of fitness or HRT on fibrinogen. Overall, physical fitness, as evaluated by aerobic capacity, was more strongly associated with fibrinolysis and coagulation than HRT. The most favorable fibrinolytic and coagulation profiles were present in the fit women regardless of HRT use, suggesting that HRT did not associate with fibrinolysis and coagulation in these fit postmenopausal women.

Previous investigations on physical fitness and hemostasis in postmenopausal women reported enhanced resting fibrinolysis in active compared with sedentary women, suggesting a positive effect of fitness on fibrinolysis (11, 41). However, both
studies also reported strong correlations between the fibrinolytic variables and BMI, WHR, and/or body fat. Thus, when these variables were controlled, fibrinolysis was no longer different between fit and unfit women or between young and old women (11). This would suggest that the difference between fit and unfit women was primarily due to the lower BMI and body size of fit women and not due to fitness per se. Similarly, the negative effect of aging on fibrinolytic variables was accounted for by the increased BMI and body size of the older women and not by fitness. Thus physical fitness per se did not prevent the age-associated decrement in fibrinolysis, but fitness prevented the age-associated increase in body weight, which in turn accounted for the improved fibrinolysis in the fit women (11). Our results support and extend these previous findings (11, 41). We found that resting levels of both tPA and PAI-1 antigen were not different between fit and unfit women after accounting for differences in BMI. However, in contrast to the findings of DeSouza et al. (11), we found that tPA activity levels were higher and PAI-1 activity levels were lower in fit women, even after accounting for differences in BMI. This disparity may be explained by HRT use. As shown in Fig. 1, HRT was associated with increased tPA activity and decreased PAI-1 activity in the women on HRT, which created vastly different profiles compared with the unfit women not on HRT. However, HRT alone does not explain our results. To further examine the influence of fitness and the possible modulating effects of body composition on resting tPA and PAI-1 activity, we conducted an analysis of covariance using just the fit women who were not on HRT while controlling for both BMI and percent body fat. This analysis showed (data not shown) that fit women still had higher resting tPA and lower PAI-1 activity compared with unfit women ($P < 0.05$). Thus our data are consistent with the notion that physical fitness positively impacts resting levels of fibrinolysis in postmenopausal women independent of body composition.

A unique aspect of our study was that we explored the fibrinolytic response to acute exercise. Maximal exercise enhances fibrinolysis by increasing tPA activity and decreasing PAI-1 activity (33, 45) and may detect impairments in the fibrinolytic system not evident at rest. Although existing research has not explored the effects of acute maximal exercise on fibrinolytic variables in postmenopausal women, our results are similar to existing data on other populations (33, 45). Szymanski et al. (44, 45) found that tPA activity was not different at rest between active and inactive men, but the active men exhibited a significantly greater increase in tPA activity after maximal exercise. Similar results were also noted after submaximal exercise, although the groups were not as highly differentiated as after maximal exercise (44). However, submaximal exercise did not decrease PAI-1 activity in either group (44), whereas maximal exercise produced a 19% decrease in PAI-1 activity in the inactive group compared with a 51% decrease in the highly active group (45). These data show the efficacy of using maximal exercise to evaluate the fibrinolytic potential. Data on patients with CVD also support the value of using maximal exercise testing to detect fibrinolytic differences in fit and unfit patients. The increase in fibrinolysis after maximal exercise has been reported to be blunted in CVD patients (3, 16); however, when CVD patients were matched on age and fitness with a healthy cohort, there were no differences in either the tPA or PAI-1 activity responses (17). Our present data support these findings and show that the fit women increased tPA activity and antigen and decreased PAI-1 activity more than the unfit women. These differences were not dependent on HRT (as similar results were seen for both fit groups, with or without HRT therapy) nor were they dependent on BMI, since adjusting for BMI did not alter these findings.

Our findings are consistent with existing research showing that HRT is associated with favorable fibrinolytic profiles (18, 26, 37), with higher resting tPA and lower resting PAI-1 activities seen in HRT users compared with nonusers. However, physical fitness influences these results, because the fit women in our study had similar fibrinolytic profiles at rest compared with both fit and unfit women on HRT. We also hypothesized that HRT would only improve the fibrinolytic response to exercise in fit women, whereas the exercise response in the unfit women would be reduced. However, the fibrinolytic response to acute exercise was independent of fitness in the groups on HRT. There was no difference in the exercise response of either tPA and PAI-1 activity between fit and unfit women on HRT, in contrast to the differences found between fit and unfit women not on HRT. Although the decrease in PAI-1 activity was not significant in the fit women on HRT, this was probably a function of the very low PAI-1 activity levels exhibited by these women and the fact that the mean PAI-1 activity dropped to close to zero after maximal exercise in this group. These findings are different from what has been reported in young women using oral contraceptives, where the fibrinolytic response to exercise was reduced in users compared with nonusers (33), suggesting that HRT and oral contraceptives exhibit different effects on the fibrinolytic system. Another important finding of our present study was that the positive associations between physical fitness and HRT on fibrinolysis were not additive, since the fit women not on HRT exhibited similar fibrinolytic profiles to the fit women on HRT. Interestingly, the unfit women on HRT also were not different from the fit women not on HRT, suggesting that the poor fibrinolytic profiles of the unfit women not on HRT may be mitigated to a similar extent by either fitness or HRT.

F1+2, a peptide liberated with activation of the coagulation system, was measured in this study as a marker of coagulation...
activation. Investigations suggest that HRT increases F1+2 (8, 37), but no effect has been reported (10). Higher levels are also seen in oral contraceptive users (33). In the present study, significantly lower F1+2 was seen in the physically fit women, regardless of HRT status, even after statistical adjustment for BMI. This is a favorable modification since elevated F1+2 is linked with increased CVD risk (31). In addition, the fit women had significantly lower F1+2 than the unfit HRT users. This is an important difference between these two groups, since they had similar fibrinolytic profiles. F1+2 levels did not increase after maximal exercise, similar to other published results using brief periods of exercise such as a maximal exercise test (33).

Neither physical fitness status nor HRT affected fibrinogen in this study. HRT has been reported to decrease (10), prevent an increase over time (14a), or produce no change in fibrinogen. There is also no consensus on the effects of physical fitness on fibrinogen. In agreement with our data, Stevenson et al. (41) reported similar fibrinogen levels in active and inactive postmenopausal women without subgroup analysis for HRT use. In contrast, DeSouza et al. (12) reported that both physical activity and HRT status affected fibrinogen with lower levels in active women and HRT users. Although there is no clear explanation for the disparate results, it is possible that differences in fitness and BMI between the studies could offer a partial explanation. Compared with the present study, the unfit women had similar fitness levels (mean maximal oxygen consumption = 24.6 vs. 24.5 ml·kg⁻¹·min⁻¹), but their fit women were more highly fit (43.5 vs. 36.6 ml·kg⁻¹·min⁻¹), which may account for the different results. Fibrinogen levels in their unfit women were fairly similar to ours (2.92 vs. 3.06 g/l), but their fit women had lower levels (2.48 vs. 2.87 g/l). The fit postmenopausal women in DeSouza et al.’s study also had a slightly lower BMI than the fit women in our study, which may also have contributed to the difference in findings.

Rankinen et al. (34) found a significant inverse relationship between fibrinogen and self-reported physical activity in postmenopausal women. However, BMI was a strong predictor of fibrinogen, and the difference between active and inactive women was only significant in the women with a BMI over 29.7. In fact, fibrinogen levels were similar in women with lower BMIs who exercised four times or more per week compared with those who exercised zero to one time per week. Data from the Postmenopausal Estrogen/Progesterin Intervention Study (40) also revealed that the strong relationship between fibrinogen and physical activity was dependent on BMI. This apparent effect of BMI on fibrinogen may help explain the lack of a difference between physically fit and unfit women in the present study. The average BMI in our subjects was lower than 29.7 (inactive = 28.0; active = 21.9), and none of the subjects’ physical characteristics significantly correlated with fibrinogen. Because fibrinogen is an independent risk factor for CVD (25), much more research is needed, particularly longitudinal, to determine whether regular exercise favorably alters fibrinogen.

Our findings may have significant implications regarding cardiovascular risk in postmenopausal women. tPA antigen is a predictor of future myocardial infarctions and may also be a marker of subclinical atherosclerosis (24, 35). Both the development of atherosclerosis and long-term risk of mortality have been related to tPA antigen and activity as well as PAI-1 activity (20, 24, 30, 36). Furthermore, a reduced tPA antigen response to maximal exercise appears to be predictive of future cardiovascular events in patients with angina pectoris (21).

An important and interesting finding of this study is that physical fitness is associated with reduced CVD risk from hemostatic factors in postmenopausal women. Overall, favorable fibrinolytic profiles and decreased coagulation activation were observed in physically fit women compared with their unfit counterparts. This is relevant because impaired fibrinolytic activity, manifesting as low tPA activity and elevated PAI-1 activity, PAI-1 antigen, and tPA antigen, has been linked with increased risk of CVD (20, 21, 23, 24) and deep vein thrombosis (32). The better fibrinolytic profile in fit women was independent of HRT; however, HRT also improved the overall profiles. Although these changes are thought to partially explain the cardioprotective effect of HRT documented in observational studies, recent studies suggest that HRT may be pro-thrombotic (22, 35a). Thus other options for risk-factor modification are needed in women with CVD and in women in whom HRT is contraindicated or controversial, including women with existing heart disease, preexisting hypercoagulable states, such as Factor V Leiden, Protein C, or S deficiency, or history of deep vein thrombosis. Improvement in physical fitness may be one such option.

The major limitation of the present study is the cross-sectional design and the possibility that preexisting factors may have influenced our findings. Therefore, it is important to interpret our findings with caution, because our findings are indicative of associations, and our data cannot be interpreted as cause and effect. Thus it is important that future longitudinal studies be conducted to confirm our findings from this cross-sectional design. However, this study was conducted to serve as pilot data for a longitudinal study. Few longitudinal studies have examined the effects of regular exercise on hemostasis in postmenopausal women. Either beneficial alterations in fibrinolytic variables (9) or statistically insignificant changes (38) have been reported. However, subjects have not been specifically subdivided according to HRT use. Because the effect of regular exercise on hemostasis in postmenopausal women has not been systematically evaluated in HRT users and nonusers, we believed it was appropriate to purposefully compare the groups before initiating a longitudinal study.

In conclusion, increased physical fitness is associated with risk-reducing hemostatic profiles in postmenopausal women, which may lead to significant health benefits. Perhaps more important, these benefits were particularly obvious in women not taking HRT, suggesting that healthy postmenopausal women either unable or unwilling to take HRT may be able to improve their hemostatic risk profile by becoming physically fit. Although we cannot state that this translates into an absolute decrease in CVD, available data suggest that these changes may predict a decrease in risk.

GRANTS

This study was supported in part by a grant from The Life Fitness Academy.

REFERENCES


