Hemostatic response to postprandial lipemia before and after exercise training

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The hemostatic response to postprandial lipemia before and after exercise training. J Appl Physiol 101: 316–321, 2006. First published February 23, 2006; doi:10.1152/japplphysiol.01363.2005.—Chronic hypertriglyceridemia is thought to be atherogenic and is associated with an elevated thrombotic potential, both of which may be improved with aerobic exercise training. Eight subjects were tested for aerobic capacity, body composition, and postprandial lipemia (PPL), followed by 6 mo of exercise training and final testing. Blood samples were obtained for measurement of free fatty acid (FFA), triglycerides (TG), insulin (Ins), and glucose (Glu). Hemostatic variables including factor VII (FVII), tissue factor pathway inhibitor-factor Xa complex (TFPI/Xa), and plasminogen activator inhibitor-1 (PAI-1) antigen/activity as well as leukocyte tumor necrosis factor-α (TNF-α) gene expression were determined among four subjects. We found that the exercise training was of sufficient intensity to increase aerobic capacity (P < 0.0001) and improve body composition (P = 0.04). There were no differences between tests among PPL responses of FFA, TG, Ins, or Glu; however, the mean TG response and fat oxidation rate improved. PAI-1 antigen/activity, FVIIa, TFPI/Xa, and TNF-α gene expression were all improved after exercise training after adjusting for confounders. We conclude that aerobic exercise training reduces the potential for coagulation, improves fibrinolytic potential, and reduces leukocyte TNF-α gene expression after the ingestion of a high-fat meal.

Atherosclerosis is now considered to be a low-grade inflammatory disease that results in endothelial cell dysfunction. Recent evidence has shown that hypertriglyceridemia, whether chronic or postprandial, is associated with leukocyte activation and an elevated potential for thrombosis, thereby promoting endothelial cell dysfunction and vascular damage (38, 39). Although the underlying mechanisms are not completely understood, it has been shown in leukocyte and endothelial cell cultures incubated with triglyceride (TG)-rich chylomicrons and very low-density lipoproteins that free fatty acid (FFA) and TG uptake activates factor VII (FVII), tissue factor (TF) (16, 21), and plasminogen activator inhibitor-1 (PAI-1) (7, 16, 35).

Studies examining the acute effects of a high-fat meal on coagulation and fibrinolysis have shown that TF and FVII activation increases (34) with an unexplained paradoxical decrease in PAI-1 levels after the meal (30, 31). This may be explained by two factors: first, after the meal, hepatic blood flow increases, which increases the clearance rate for PAI-1; and second, PAI-1 concentrations are highest in the morning, but because of diurnal variations, they decrease throughout the day. Taken at face value, these results suggest that, although a high-fat meal increases the potential for thrombosis, there is an inherent cardioprotective effect because of the increased fibrinolytic potential. Therefore, the purpose of this study was to measure, in addition to plasma protein concentrations, PAI-1, interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) gene transcription in leukocytes after the high-fat meal.

Additionally, exercise training improves a wide range of cardiovascular disease (CVD) risk factors; however, the results of studies examining the effects of exercise training on inflammation, coagulation, and fibrinolysis have been inconsistent (9, 11, 20, 37, 41, 42). This may be due to the fact that the majority of previous studies measured the various hemostatic markers during resting or fasting conditions. This would not give a complete picture of the capacity of either system because the hemostatic mechanisms are designed to activate in response to a stimulus. Our group and others have found that after a high-fat meal, FFA and TG clearance is improved after exercise training (4, 29), and these results lead to the hypothesis that the reduction in FFA and TG levels after exercise training will reduce inflammation and coagulation and increase fibrinolysis after a high-fat meal.

METHODS

The study protocol and informed consent was approved by the University of Maryland College Park Institutional Review Board, and each subject reported to the laboratory and their informed consent was obtained. Subject inclusion criteria and screening methods have been defined previously (22, 25), however briefly; sedentary 50- to 75-yr-old men and women who were nonsmokers, did not have diabetes, were not on lipid-, glucose (Glu)-, or blood pressure-lowering medications, and were not receiving anticoagulant therapies were included in the study. All subjects maintained an American Heart Association low-fat diet (3) verified by food records throughout the baseline testing, exercise training, and final testing phases of the protocol.

Subjects were free from infection, fever, cold, or any other illness for at least 1 wk and abstained from taking any medications (including aspirin or other nonsteroidal anti-inflammatory drugs), vitamins, herbal supplements, and/or alcohol for 2 days before the postprandial lipemia test (PPLT). For the baseline PPLT, subjects did not engage in physical activity for at least 5 days before testing, because acute exercise has been shown to affect FFA and TG clearance (2, 12, 14). For final PPLT testing, subjects performed their last bout of exercise 24–36 h before the PPLT because the inclusion of acute exercise more accurately represents their current daily condition during exercise training.

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Subjects reported to our laboratory for testing and consumed the standard liquid fat meal (24) within 3 min between 0700 and 0900 to minimize the effect of diurnal variation on the markers of inflammation, coagulation, and fibrinolysis. Expired gases were collected into Douglas bags in 5-min intervals for a total of 20 min before meal ingestion, and at 2 and 4 h after meal ingestion. Each 5-min bag was analyzed for gas content (O2, CO2, and N2) using a medical gas analyzer (Perkin Elmer, Danbury, CT) and volume. Respiratory exchange ratio, carbohydrate (CHO) and lipid oxidation rates, and total energy expenditure (TEE) were estimated using previously established criteria (10).

Blood samples were obtained before ingestion of the fat meal and every 30 min for 4 h after the meal for the determination of plasma metabolic factors [insulin (Ins), Glu, FFAs, and TG]. Serum and EDTA plasma were analyzed for changes in FFA (NEFA C, Wako Chemicals, Richmond, VA), TG (Sigma Diagnostics, St. Louis, MO), Ins (Linco Research, St. Charles, MO), and Glu (YSI 2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, OH) concentrations. High sensitivity C-reactive protein (hsCRP) (Alpha Diagnostic International, San Antonio, TX), tissue factor pathway inhibitor-factor Xa complex (TFPI/Xa), tissue factor pathway inhibitor-factor Xa complex (TFPI/Xa; Imubind, American Diagnostics, Greenwich, CT), and PAI-1 Ag (Zymutest, Diapharma, West Chester, OH) were measured by enzyme-linked immunosorbent assay and PAI-1 activity (Spectrolyte PL, American Diagnostica) was measured by an amylohexylic activity assay. All samples were measured in duplicate within the same run to minimize variation among samples.

Whole blood gene expression. RNA was extracted according to the manufacturer's recommendation (PaxGene Blood RNA Kit, Qiagen, Valencia, CA) and using the optional on-column DNase treatment (RNase-free DNase set, Qiagen). A total of 100 ng of RNA was used for the reverse transcription reaction, and real-time PCR was carried out using a Roche Lightcycler (Roche, Mannheim, Germany) for PAI-1, IL-6, TNF-α, and RNA polymerase II (RNA pol II) mRNA. Relative expression was estimated using the cycle threshold method (13).

Exercise training intervention. All subjects underwent three exercise training sessions per week supervised by study personnel. The training program lasted 6 mo to ensure adequate time for training-induced improvements in the cardiovascular and metabolic systems. Initial training sessions consisted of 20 min of exercise at 50% maximal O2 uptake (VO2 max) and increased by 5 min every week until 40 min of exercise at 50% VO2 max were completed each session. Training intensity then increased by 5% VO2 max every week until an intensity of 70% VO2 max was achieved. Subjects added a lower intensity unsupervised 45–60 min walk on the weekend after 10 wk of training and recorded in printed logs their exercise heart rate, duration, and mode information for all supervised and unsupervised training sessions. Adherence to the training prescription was assessed for every exercise training session by inspecting training log exercise intensity, duration, and frequency data.

Final testing. At the completion of the exercise training intervention, subjects completed 7-day food records to ensure dietary compliance before reassessment of all baseline measures. Subjects continued their exercise training until all final testing was completed. Each subject underwent the final postprandial lipemia test 24–36 h after their last exercise session.

Statistics. A two-factor (test ˟ time) repeated-measures ANOVA using a heterogeneous-compound symmetry matrix was used to analyze the interaction effects for changes in plasma coagulation and fibrinolytic variables, as well as leukocyte TNF-α gene expression. Statistical analyses were performed using SAS software (SAS version 8.2, SAS Institute, Cary, NC). The a priori alpha level was set at P < 0.05 for all planned comparisons. Analysis of the residual variance was conducted to ensure a normal distribution was present. Potential covariates for the selected variables measured in this study include body composition measures (total fat and intra-abdominal fat), fasting lipoproteins [low-density lipoprotein (LDL), high-density lipoprotein (HDL) subfraction 3], and postprandial lipemic measures (hsCRP, FFA, Ins, and lipid oxidation rate). A maximum of four potential covariates were included for each variable; thus only the most biologically reasonable variables were used. Nonsignificant (β = 0.05) covariates were removed from the model one at a time, starting with the least significant and ending when all remaining were significant.

RESULTS

Exercise training intervention. A total of eight subjects completed all aspects of the exercise training intervention and PPLT testing. Although the intervention was of sufficient frequency, intensity, and duration to elicit improvements in VO2 max, body composition, and total cholesterol, there were no improvements in fasting hsCRP, TG, LDL, HDL, or HDL subfractions (Table 1) with 6 mo of training. All subjects maintained initial total body weight throughout the intervention only experiencing a nonsignificant decrease in body mass at final testing (~1.3 kg).

Postprandial lipemia test. There were no differences between fasting measures of FFA, TG, Ins, or Glu at baseline vs. final testing. There was a significant increase in plasma FFA, TG, and Ins levels during the PPLT at 2 and 4 h after meal consumption before and after exercise training (Table 2). There were no significant differences in the postprandial levels at each time point between the two tests, although the time averaged mean postprandial TG levels were lower at final testing (139 ± 19 mg/dl) vs. baseline (154 ± 24 mg/dl) (P = 0.02). There was no significant difference between time averaged FFA (P = 0.27) or Ins (P = 0.17) levels between the two tests. Plasma Glu levels remained unchanged throughout each test. However, the average CHO oxidation rate across the entire 4-h postprandial lipemia test significantly decreased from 282 ± 48 to 201 ± 26 mg/min (P = 0.009) and total lipid oxidation during the same period increased from 10.5 ± 13.1 to 32.8 ± 9.3 mg/min (P = 0.01), whereas average TEE remained unchanged (1.14 ± 0.05 vs. 1.07 ± 0.09 kcal/min; P = 0.29) between the two tests.

Plasma coagulation and fibrinolytic measures. Before exercise training, log10 PAI-1 Ag levels significantly decreased from fasting to 2 h postprandial with a nonsignificant trend

Table 1. Fasting subject characteristics before and after exercise training

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Final</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>58.9 ± 4.7</td>
<td>58.6 ± 4.4</td>
<td>0.007</td>
</tr>
<tr>
<td>VO2 max, l/min</td>
<td>2.2 ± 0.2</td>
<td>2.6 ± 0.2</td>
<td>0.007</td>
</tr>
<tr>
<td>Total fat, kg</td>
<td>30.0 ± 3.6</td>
<td>28.8 ± 3.7</td>
<td>0.04</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>80.4 ± 3.6</td>
<td>79.1 ± 3.6</td>
<td>0.80</td>
</tr>
<tr>
<td>hsCRP, mg/l</td>
<td>0.8 ± 0.6</td>
<td>0.96 ± 0.6</td>
<td>0.51</td>
</tr>
<tr>
<td>TC, mg/dl</td>
<td>194 ± 13</td>
<td>169 ± 10</td>
<td>0.004</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>109 ± 13</td>
<td>99 ± 12</td>
<td>0.56</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>49 ± 6</td>
<td>45 ± 5</td>
<td>0.64</td>
</tr>
<tr>
<td>HDL2, mg/dl</td>
<td>8.9 ± 3.1</td>
<td>8.1 ± 2.8</td>
<td>0.85</td>
</tr>
<tr>
<td>HDL3, mg/dl</td>
<td>40.2 ± 4.1</td>
<td>37.3 ± 2.5</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Values are means ± SE for 8 subjects. Baseline: before aerobic exercise training; Final: after 6 mo of aerobic exercise training; VO2 max: maximal O2 uptake; hsCRP, high-sensitivity C-reactive protein; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HDL2, and HDL3, HDL subfractions 2 and 3, respectively.
Table 2. Fasting and postprandial metabolic variables before and after exercise training

<table>
<thead>
<tr>
<th>Test</th>
<th>Fasting</th>
<th>2 h</th>
<th>4 h</th>
<th>Mean Differences Within Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG, mg/dl</td>
<td>102±20</td>
<td>155±27</td>
<td>222±33</td>
<td>0 h&lt;2 h&lt;4 h</td>
</tr>
<tr>
<td>Final</td>
<td>93±17</td>
<td>135±18</td>
<td>196±28</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.48</td>
<td>0.25</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>FFA, mmol/l</td>
<td>0.36±0.05</td>
<td>0.30±0.04</td>
<td>0.53±0.05</td>
<td>0 and 2 h&lt;4 h</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.40±0.07</td>
<td>0.31±0.05</td>
<td>0.54±0.04</td>
<td>0 and 2 h&lt;4 h</td>
</tr>
<tr>
<td>P value</td>
<td>0.55</td>
<td>0.90</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>67±10</td>
<td>180±38</td>
<td>136±26</td>
<td>0 and 4 h&lt;2 h</td>
</tr>
<tr>
<td>Baseline</td>
<td>69±12</td>
<td>162±39</td>
<td>118±18</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.86</td>
<td>0.63</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>98±3</td>
<td>106±6</td>
<td>98±4</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline</td>
<td>98±3</td>
<td>105±5</td>
<td>105±5</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.90</td>
<td>0.88</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>CHOox, mg/min</td>
<td>298±66</td>
<td>301±55</td>
<td>248±40</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline</td>
<td>198±34</td>
<td>203±21</td>
<td>203±31</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.04</td>
<td>0.03</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Lipidox, mg/min</td>
<td>2±20</td>
<td>−2±2</td>
<td>31±9</td>
<td>2 h&lt;4 h</td>
</tr>
<tr>
<td>Baseline</td>
<td>26±8</td>
<td>33±12</td>
<td>39±13</td>
<td>NS</td>
</tr>
<tr>
<td>P value</td>
<td>0.16</td>
<td>0.02</td>
<td>0.30</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE for 8 subjects. TG, triglycerides; FFA, free fatty acids; CHOox, carbohydrate oxidation rate; Lipidox, lipid oxidation rate; NS, no significant difference. P value represents mean comparisons between tests at each level of time (i.e., fasting baseline vs. fasting final). Mean differences within test represents a significant difference between time points within a single test (i.e., fasting baseline vs. 2-h baseline vs. 4-h baseline).

toward a further reduction from 2 to 4 h, and at 4-h postprandial, log_{10} PAI-1 Ag levels were significantly lower than fasting (Table 3). After exercise training, log_{10} PAI-1 Ag levels did not change significantly from fasting to 2 h but decreased from 2 to 4 h, and at 4 h the levels were lower than fasting. At final testing, log_{10} PAI-1 Ag levels were lower in the fasted state, not significantly different at 2 h, and significantly lower at 4-h postprandial vs. baseline.

There was no change in PAI-1 activity from fasting to 2-h postprandial before or after exercise training after adjusting for intra-abdominal fat and lipid oxidation rate; however, during both tests, there was a significant decrease from 2 to 4 h. There were no differences in the fasting measures of PAI-1 activity between tests; however, after exercise training, the 2-h and 4-h postprandial PAI-1 activity levels were significantly lower than the respective baseline values.

After adjusting for LDL and lipid oxidation rate, before exercise training, FVIIa levels increased from fasting to 2 h postprandial and decreased significantly from 2 to 4 h where the levels was no longer elevated compared with fasting levels. At final testing, FVIIa levels decreased significantly from fasting to 2 h, increased significantly from 2 to 4 h, and were not different from fasting at 4 h postprandial. FVIIa was not different between tests before meal ingestion; however, at 2 and 4 h, FVIIa was significantly lower at final testing compared with baseline.

TFPI/Xa levels at baseline testing did not change during the 4-h postprandial lipemia test after adjusting for LDL and FVIIa levels. However, at final testing, there was a significant increase in TFPI/Xa levels from fasting to 2 h and from fasting to 4 h, with no change from 2 to 4 h. There was no difference between tests in fasting levels of TFPI/Xa; however, at 2 and 4 h, TFPI/Xa levels were significantly higher at final vs. baseline testing.

Whole blood gene expression. Neither fluorescence nor post-PCR product was detectable for IL-6 or PAI-1 mRNA in the subject samples; however, TNF-α and RNA pol II gene expression were robust and detectable in all subject samples, and the data for all four subjects are presented in Fig. 1. After adjusting for hsCRP and total body fat mass, TNF-α gene expression increased from fasting to 2-h postprandial and decrease in TFPI/Xa levels from fasting to 2 h and from fasting to 4 h, with no change from 2 to 4 h. There was no difference between tests in fasting levels of TFPI/Xa; however, at 2 and 4 h, TFPI/Xa levels were significantly higher at final vs. baseline testing.
decreased significantly from 2 to 4 h such that the 4-h postprandial value was significantly lower than fasting before exercise training. At final testing, TNF-α gene expression increased from fasting to 2 h and remained significantly elevated at 4 h vs. the fasting level. Between tests, fasting TNF-α gene expression was lower at final testing, lower at 2-h postprandial, and not different at 4 h vs. baseline.

**DISCUSSION**

**Exercise training intervention.** The present study was designed to assess the changes in the hemostatic response to a postprandial lipemia test before and after 6 mo of aerobic exercise training. Whereas there was no control group in which to compare random and/or seasonal changes among the selected variables, we were able to compare each subject’s baseline value with the change after the stimulus. We also sought to minimize the amount of weight lost during the study intervention, because weight loss has been shown to affect hemostasis and inflammation independent of metabolic and cardiovascular adaptations (33, 36). Despite the fact that there were no demonstrated improvements in lipoprotein substraction or fasting Ins levels, the exercise training intervention was of sufficient intensity to elicit significant improvements in aerobic capacity and body composition.

**Postprandial lipemia test.** The standard fat meal employed in the present study design sufficiently induced a lipemic response; however, there were no differences between tests (baseline vs. final) among postprandial values for FFA, TG, Ins, or Glu at any single time point (0, 2, or 4 h). There was a significant reduction in the average TG response across all time points from baseline to final testing, which is in agreement with previous studies (12, 14). Others have reported significant improvements in the postprandial lipemic response after acute bouts of physical activity, with the majority of the improvements observed within 12–24 h after exercise. Our study assessed the response at 24–36 h after the last bout of exercise, which may explain the fact that no differences were observed in these variables and confirms a previous 12-wk exercise training study in which there was no reported reducton in postprandial lipemia 48 h after a single bout of acute exercise (1).

The major factor influencing the improved lipemic response after exercise training is believed to be an increase in lipoprotein lipase activity that has been shown to be transient, with the largest increase in activity occurring within 18 h after an acute bout of exercise (17, 29). Another and less transient factor that is associated with aerobic exercise training is an improved skeletal muscle β-oxidative capacity, and we observed a significant decrease in the average rate of CHO oxidation with a concomitant increase in lipid oxidation during the postprandial lipemia test after exercise training. Unlike the transient nature of skeletal muscle LPL activity, the increase in β-oxidative capacity remains elevated for days or weeks with chronic aerobic exercise training. We hypothesized that the exercise training-mediated improvements in inflammation and hemostasis would be due to improvements in FFA and TG clearance; however, the results of this study imply that the improvements may be due to increased FFA oxidation.

It is known that the tissue type involved in the uptake and clearance of FFA, TG, and Glu from plasma may contribute to the inflammatory and hemostatic response to an oral fat load (18). In the sedentary state, more of the ingested TG is directed to adipocyte storage, which has been shown to increase the expression of inflammatory cytokines and PAI-1 levels (40). Thus, with a larger amount of the ingested fat being directed toward β-oxidation in the trained state, adipocyte-directed storage and cytokine/PAI-1 expression would be decreased.

Finally, it is not unreasonable to assume that with such a large amount of ingested fat in a single meal, enterocyte chyomicron synthesis and secretion may be occurring at near maximal capacity. In this case, the postprandial levels of plasma FFA, TG, and Ins would not change over a 4-h time period before vs. after exercise training due to the continued enterocyte FFA uptake, chyomicron secretion, and plasma appearance of TG. Thus the ability to determine changes in plasma FFA and/or TG clearance may be delayed until complete intestinal clearance of the ingested meal has occurred. Many previous studies have assessed the postprandial lipemic response for ≥8 h and have shown that the peak lipemic response tends to be at 4 h. It is therefore possible that differences between time points may exist beyond the peak response, although the usefulness of measurements beyond 4 h is in question as people seldom go more than 4 h between meals.

**Plasma coagulation and fibrinolytic measures.** One of the purposes of this investigation was to more accurately define the effect of aerobic exercise training on hemostasis by measuring the response of the system under stress. The majority of previous studies that have attempted to show a reduction in coagulation potential after long-term engagement in physical activity or exercise training have done so using an assessment of coagulation factors in the fasted state and have failed to show a clear and consistent reduction (9, 20, 37, 41, 42). This is likely due to the fact that the hemostatic response functions over a wide range and that many of the individual factors are capable of increasing their activity to more than 100% over resting levels (23). It would be unreasonable to assume that a clear reduction could be apparent when assessing the system within the lowest range of its activity. This is confirmed by the results of the present study in which the levels of FVIIa and TFPI/Xa showed no differences in the fasted state between tests (baseline vs. final), yet there was a clear difference between tests after the fat meal.

There is very little known about the effect of exercise training on anticoagulant activity, with only one study published in which TFPI levels were measured before and after exercise training where the authors reported no change in fasting levels of circulating TFPI among diabetic subjects (28). However, circulating TFPI is not believed to be a good predictor of functional or total TFPI, which is why we chose to measure the TFPI/Xa complex. The majority of functional TFPI is believed to be bound to the endothelium where it is capable of binding to the TF/FVIIa/Xa ternary complex and rapidly terminating the activation of coagulation. Very little is known about the effect of exercise training on plasma TFPI levels, and nothing is known about changes in endothelial cell expression of TFPI with training. Here we show an increase in TFPI-mediated inactivation of the extrinsic pathway after training, and although it is beyond the scope of this investigation, it is tempting to speculate that a higher quantity
of TFPI may have been expressed on the endothelial cell surface after training.

As with coagulation potential, previous studies on the effect of exercise training on fibrinolytic potential have reported either decreases or no change in PAI-1 antigen or activity levels (15, 32). The two most logical reasons for the disagreement between these previous results are 1) variation in exercise training frequency, intensity, and/or duration, and 2) measurement of fasting PAI-1 levels. In the present study, we employed a long-term, relatively high-intensity exercise training program and we measured the response of PAI-1 after a meal challenge. As a result, we were able to illustrate a clear reduction in postprandial levels of PAI-1 Ag and activity despite the fact that there was no significant difference in fasting PAI-1 activity levels with training. These results further illustrate the fact that assessing the hemostatic response after a challenge improves the ability to observe change above that which is seen in the fasted state.

Another main outcome of this project was to address the previously noted paradoxical decrease in PAI-1 levels during the postprandial lipemia test. No study to date has attempted to address this discrepancy, which taken at face value suggests that fibrinolytic activity is increased after a high-fat meal. The most logical explanation for the resulting decrease in PAI-1 Ag is that the rate of hepatic blood flow is higher after ingestion of the meal. In addition to this, PAI-1 activity levels may be decreasing, at least in part, due to diurnal variations with the highest levels of activity in the morning. In light of these facts, it is possible that endothelial, hepatic, or adipocyte PAI-1 release may in fact increase while plasma levels are decreasing. Despite the fact that this paradoxical PAI-1 decrease exists after a fat meal, the fact that postprandial PAI-1 Ag and activity were lower after exercise training in the present study illustrates a clear benefit of aerobic exercise training on reducing the risk for CVD-related outcomes.

Whole blood gene expression. We sought to examine the effect of exercise training on the postprandial response of leukocyte gene expression after a high-fat meal. Our aim was to determine whether PAI-1 and inflammatory gene expression were increased despite the paradoxical decrease in circulating PAI-1 protein levels. Unfortunately, we were unable to address this aspect of the investigation, most likely due to the fact that monocytes comprise a small fraction of the whole blood leukocyte population (0–9%), and without separation of cell types before RNA isolation, they provide an equally low contribution of mRNA to the total mRNA obtained. It was understood at the onset of the investigation that monocytes would be the only cells expressing IL-6 and PAI-1 (19); however, we believed that their mRNA would still be detectable even in the presence of whole blood leukocyte mRNA. Based on the results of this study, and others (5, 6, 8, 26), it is apparent that an investigation of monocyte-derived mRNA should be conducted only after separation of mononuclear cells and whole blood gene expression should be avoided.

In addition to monocytes, TNF-α is expressed in multiple mononuclear blood cell types, including B cells, T cells (43), and neutrophils (27, 43). Consequently, its expression was robust in our samples and we found a substantial reduction in TNF-α gene expression, which would indicate an improvement in inflammation at rest and after a meal challenge due to aerobic exercise training. Whereas the high-fat meal increased leukocyte TNF-α gene expression from fasting to 2-h postprandial during both tests, the degree to which gene expression increased in the trained state was lower than before training. We are unable to determine whether the observed changes in TNF-α gene expression resulted in comparable changes in plasma TNF-α levels; nevertheless, it is reasonable to conclude that the leukocyte TNF-α response to the high-fat meal was lower after training vs. baseline.

In summary, given that the majority of life is spent in the postprandial state and because the inflammatory and hemostatic systems function under a wide range of values in response to stimuli, assessment of their function in the fasted state does not provide an accurate description of the effect of exercise training. However, the results of this investigation provide clear evidence that inflammation and the coagulation and fibrinolytic potentials are improved with aerobic exercise training. In addition, by reducing or eliminating the increase in coagulation potential and inflammation and increasing fibrinolysis, aerobic exercise training may reduce the risk for CVD and stroke-related morbidity and mortality. Finally, this is the first investigation into the effect of exercise training on anti-coagulant potential among healthy subjects, and future studies into the area of exercise and hemostasis should focus on assessment of the systems under a controlled stress while determining the change in TFPI, antithrombin III, and protein C activities in addition to procoagulant changes.

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REFERENCES


