Effect of endurance training and seasonal fluctuation on coagulation and fibrinolysis in young sedentary men


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Van den Burg, P. J. M., J. E. H. Hospers, M. Van Vliet, W. L. Mosterd, B. N. Bouma, and I. A. Huisveld. Effect of endurance training and seasonal fluctuation on coagulation and fibrinolysis in young sedentary men. J. Appl. Physiol. 82(2): 613–620, 1997.—The effect of 12 wk of submaximal training on hemostatic variables was studied in 20 young sedentary men (Tr) and 19 nontraining matched controls (Con). After training, a more pronounced increase in factor VIII coagulant activity (P < 0.01), reflected in a decrease in activated partial thromboplastin time (P < 0.01) during maximal exercise, was seen. Both basal plasminogen activator inhibitor 1 antigen (PAI-1Ag) and activity (PAI-1Act; P < 0.05), together with an increase in basal and exercise-induced tissue-type plasminogen activator antigen (t-PA Ag; P < 0.05), were decreased after training. The overall effect on fibrinolysis was reflected in an increase in the t-PAAct/t-PA Ag ratio in the Tr group. In contrast, during the same period (February-June), the Con group demonstrated an increase in basal PAI-1Ag (P < 0.01), as well as basal and exercise-induced tissue-type plasminogen activator antigen (t-PA Ag; P < 0.05), reflected in a decrease in activated partial thromboplastin time (P < 0.01) during maximal exercise, was seen. Both basal plasminogen activator inhibitor 1 antigen (PAI-1Ag) and activity (PAI-1Act; P < 0.05), together with an increase in basal and exercise-induced tissue-type plasminogen activator antigen (t-PA Ag; P < 0.05). Both basal and exercise-induced tissue-type plasminogen activator antigen (t-PA Ag; P < 0.05) were decreased after training. The overall effect on fibrinolysis was reflected in an increase in the t-PAAct/t-PA Ag ratio in the Tr group. In contrast, during the same period (February-June), the Con group demonstrated an increase in basal PAI-1Ag (P < 0.01), as well as basal and exercise-induced tissue-type plasminogen activator antigen (t-PA Ag; P < 0.05), were decreased after training. The overall effect on fibrinolysis was reflected in an increase in the t-PAAct/t-PA Ag ratio in the Tr group. In contrast, during the same period (February-June), the Con group demonstrated an increase in basal PAI-1Ag (P < 0.01), as well as basal and exercise-induced tissue-type plasminogen activator antigen (t-PA Ag; P < 0.05), were decreased after training. The overall effect on fibrinolysis was reflected in an increase in the t-PAAct/t-PA Ag ratio in the Tr group.
of sporting activity during leisure time. Other inclusion criteria were no sporting activities during the previous five years, apparent health, no medication, being a nonsmoker, and only moderate alcohol use. Forty participants were selected and randomly divided into a trained group (Tr; n = 20) and a Con group (n = 20). The study was approved by the Ethical Commission of the Utrecht University Hospital, and participants joined the study after written informed consent was obtained.

Training and Test Procedure (Fig. 1)

Training. The Tr group participated for 12 wk (February-June) in supervised training sessions that were performed in an exercise room in the laboratory. Participants exercised twice a week for 1 h at a constant submaximal level. The work rate was adjusted continuously for each individual during each training session to maintain a heart rate corresponding with that at 60–70% VO\textsubscript{2max}. At this submaximal level, on the basis of recommendations for recreational sporting activities, clear training-induced changes can be expected (2).

Anthropometry and diet analysis. Height, body mass, and four skinfolds were measured as described before (3), and body mass index (BMI; kg/m\textsuperscript{2}) and fat percent were calculated.

Participants completed a 3-day food record (two weekdays and one weekend day) before the start, after 6 wk, and in the last week of the program. These data were analyzed by an experienced dietician for the macronutrients proteins, fats, carbohydrates, and fibers. The results were expressed as percentage of the total caloric intake.

The experimental design of the study is presented in Fig. 1. VO\textsubscript{2max} test. VO\textsubscript{2max} was determined with an increasing workload test on a cycle ergometer (Lode, Groningen, The Netherlands). Subjects started with an initial load of 1 W/kg (60 rounds/min), which was increased every 2 min by 1 W/kg. When a heart rate (HR) of 150 beats/min was attained, the load was increased 0.5 W/kg every 2 min until participants reached their maximal performance. Participants were encouraged to exert themselves maximally. Maximal performance was indicated by the inability to continue, predicted maximal HR (HR\textsubscript{max}) (2), and by a respiratory exchange ratio >1.15.

The total work capacity was calculated, at each step during the VO\textsubscript{2max} test, as the product of load (W = J/s) and time (s). The total amount of work (J) is expressed per kilogram of body mass. Ventilatory parameters were determined with an Oxycon-β (Mijnhardt, The Netherlands), which was calibrated before and after each test. The electrocardiograph was monitored continuously by using three leads (CC5, CM5, and CB5) with a megacart electrocardiograph (Siemens, The Netherlands). In addition, HR\textsubscript{max} and the HR at 60 and 70% VO\textsubscript{2max}, respectively, were recorded. These parameters were used for the standardization of the exercise test (Ex-test) procedure (see below) and training intensity.

Blood-collection procedure. Blood was drawn via a cannula (Vasculor 2, 18 gauge, Viggo, Sweden) that was placed in the antecubital vein. The first ml of each blood sample were voided, and the cannula was flushed with 3 ml saline after each sampling procedure. Blood was collected in tubes containing chilled 3.8% (0.11 mmol/l) trisodium citrate and in EDTA-coated tubes. For the determination of t-PA Act, 1 ml of citrate blood was immediately mixed with an equal amount of sodium acetate buffer (0.2 mol/l, pH 3.9). Blood for PAI-1 antigen (PAI-1 Ag) determinations was collected in tubes containing citric acid, theophillin, adenosine, and dipyridamol (Becton-Dickinson). Within 10 min after collection, plasma was separated by centrifugation at 2,000 g for 20 min at 4°C, divided into small aliquots of 200 µl, snap-frozen in liquid nitrogen, and stored at −80°C.

Hematologic parameters. Samples from each individual obtained before, after 6 wk, and after 12 wk of training were tested simultaneously in one run to eliminate the intra-assay variation. Each assay run comprised an equal number of Tr and Con subjects.

Coagulation activity of factor VII (FVII:c), factor VIII (FVIII:c) factor IX (FIX:c), factor XI (FIII:c), and fibrinogen (Fbg) as well as activated partial thromboplastin time (APTT) and prothrombin time were determined, according to the manufacturer’s instructions, with a laser-nephelometric centrifugal analyzer (ACL 200, Instrumentation Laboratory, Instrumentation Laboratory, Instrumentation Laboratory). Deficient plasmas were obtained from Organon Teknika Nederland (Boxtel, The Netherlands). Cephaline, calcium chloride, and calcium thromboplas-
tin were provided by Instrumentation Laboratory. Blood for the normal plasma pool was donated by 40 healthy men.

Table 1. Anthropometric and exercise characteristics of participants

<table>
<thead>
<tr>
<th>B</th>
<th>T6</th>
<th>T12</th>
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<tr>
<td>Tr, Con</td>
<td>Tr, Con</td>
<td>Tr, Con</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>76 ± 2</td>
<td>76 ± 2</td>
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<tr>
<td>BMI, kg/m²</td>
<td>22.6 ± 0.3</td>
<td>22.5 ± 0.4</td>
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<tr>
<td>Body fat, %</td>
<td>19.9 ± 0.9</td>
<td>18.4 ± 1.1</td>
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<tr>
<td>( V_\text{O}_{2,\text{max}} ), l/min</td>
<td>3.62 ± 0.09</td>
<td>3.74 ± 0.08</td>
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<td>HRmax, beats/min</td>
<td>190 ± 2</td>
<td>192 ± 2</td>
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<tr>
<td>TWC, l/kg</td>
<td>1.62 ± 0.08</td>
<td>1.73 ± 0.11</td>
</tr>
<tr>
<td>Hb, mmol/l</td>
<td>9.3 ± 0.11</td>
<td>9.4 ± 0.10</td>
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<tr>
<td>Hct</td>
<td>0.41 ± 0.01</td>
<td>0.42 ± 0.01</td>
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Values are means ± SE; n = 39 subjects. B, before training (February); T6, 6th wk of training (April); T12, 12 wk of training (June); Tr, training group; Con, control group; \( V_\text{O}_{2,\text{max}} \), maximum \( O_2 \) uptake; BMI, body mass index; HR, heart rate; TWC, total work capacity; Hb, hemoglobin; Hct, hematocrit. *Significant increase in Tr group from B to T6, \( P < 0.05 \).
efficiency, represented as the t-PA Act/Ag ratio, tended to increase in the Tr group and decrease in the Con group. Again, the effects were not significant within groups, but the differences between Tr and Con groups became significant (P < 0.05).

EXERCISE-RELATED PLASMA LEVELS (FIG. 5). Comparable changes to those observed in basal levels were seen in the exercise-related levels of t-PA Ag and t-PA Act/Ag. The percent increase in t-PA Ag during maximal performance was not changed in either the Tr (B, T6, and T12: 280 ± 33, 310 ± 34, and 291 ± 26%, respectively) or the Con groups (B, T6, and T12: 268 ± 25, 316 ± 35, and 296 ± 28%, respectively). The results indicate that the changes in basal levels, rather than the magnitude of the exercise-induced changes, are responsible for the significant differences between Tr and Con groups at T12.

Both groups demonstrated a considerably enhanced increase in u-PA Ag during maximal exercise at T6 and T12. The increase at T12 was significantly larger (P < 0.02) in the Tr group than in the Con group (not indicated in Fig. 5).

Repeatability. Coefficients of variation within series and between series were 3.0 and 5.5% (n = 10), respectively, for coagulation factors and were 4.0 and 7.0% (n = 10), respectively, for fibrinolytic components.

Effects of hemoconcentration. Changes in plasma volume during maximal performance increased over the 12-wk period in both Tr (B, T6, and T12: 11 ± 1, 12 ± 1, and 14 ± 1%, respectively) and Con groups (B, T6, and T12: 13 ± 1, 14 ± 1, and 15 ± 1%, respectively) to a comparable extent. Correction for the effect of hemoconcentration did not affect the results that were related to training or seasonal fluctuation. Uncorrected data, reflecting the in vivo situation, are presented.

DISCUSSION

Physical inactivity is associated with an almost twofold increased risk of developing coronary heart disease (4) and constitutes an important modifiable lifestyle risk factor. More recently, in a joint position statement of the World Health Organization, physical inactivity has been declared an independent risk factor for coronary heart disease (4, 21). A sedentary lifestyle may result in the development of cardiovascular complications through various pathophysiological mechanisms. Regular physical exercise has been shown to produce several beneficial effects. Favorable changes in

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<th>Table 2. Dietary analysis: macronutrients</th>
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</tr>
<tr>
<td>Energy, MJ</td>
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<td>Protein, En%</td>
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<td>Carbohydrate, En%</td>
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<td>Fiber, g/MJ</td>
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Values are means ± SE; n = 39 subjects. En%, percentage of total energy intake. No significant differences between or within Tr and Con groups at B or during training were observed.
cholesterol metabolism (36) and blood pressure regulation (33) have been reported. We were interested in the effect of moderate physical training in subjects not accustomed to any form of recreational physical exertion.

Because the precise nature of the mechanism by which physical activity exerts its protective effect is not known, we have focused our attention on components of the hemostatic balance. Coagulation plays a role in the process of clot formation, whereas fibrinolysis is responsible for clot resolution.

Beneficial effects of strenuous physical training on fibrinolytic activity in athletes have been reported (26–28, 34) predominantly in cross-sectional designs. Well-designed longitudinal studies are scarce. In contrast to the fibrinolytic system, the coagulation system has so far received little attention (27).

In the present study, the effect of physical conditioning on components of both coagulation and fibrinolysis was investigated. A Tr group of sedentary men participated in a highly standardized test and training program, whereas a group of matched men served as Con.

Acute exhaustive exercise is known to induce an increase in both coagulation and fibrinolytic activity. During the subsequent recovery period, a sharp fall in fibrinolytic activity parallel to a persistent coagulant activity is observed. This phenomenon may constitute an additional risk factor for coronary thrombosis in susceptible persons (32).

![Graphs showing changes in clotting factors during training](image-url)
The results of the present study indicate that the training-induced increase in physical fitness is associated with a significantly enhanced coagulation activity. During maximal exercise and recovery, the magnitude of the increase in FVIII:c was significantly enhanced. This enhancement was reflected in a more pronounced shortening of the APTT. A high correlation ($r = 0.58$) was observed between the training-induced increase in $\dot{V}O_{2\text{max}}$ and the training-induced changes (increase with respect to decrease) in FVIII:c and APTT during exercise. These results underscore those of Korsan-Bengtsson et al. (20) obtained in a cross-sectional study. They investigated 722 men (mean age 54 yr) and reported that individuals with a higher degree of physical activity had shorter clotting times. This training-related enhanced hypercoagulability during maximal exercise

![Graphs A, B, C](image)

Fig. 5. Tr and Con groups were tested at B (A), T6 (B), and T12 (C). Values are means ± SE for $n = 20$ subjects (Tr) and $n = 19$ subjects (Con). Acute exercise induces a significant increase in tissue-type plasminogen activator activity (t-PA Act) and antigen (t-PA Ag), t-PA Act/Ag ratio, and urokinase-type plasminogen activator antigen (u-PA Ag) at B, T6, and T12 (not shown). Symbols are defined as in Fig. 3. *Significant difference between Tr and Con in training-induced changes of fibrinolytic parameters (B vs. T6 and B vs. T12), $P < 0.05$.

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<table>
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<th>Table 3. Basal level of fibrinolytic variables</th>
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<td>PAI-1 Ag, ng/ml</td>
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<tr>
<td>PAI-1 Act, IU/ml</td>
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<tr>
<td>t-PA Ag, ng/ml</td>
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<tr>
<td>t-PA Act, IU/ml</td>
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<td>t-PA Act/Ag</td>
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Values are means ± SE; $n = 39$ subjects. PAI-1, plasminogen activator inhibitor; Ag, antigen; Act, activity; t-PA, tissue-type plasminogen activator; IU, international unit. *Significantly different between Tr and Con groups at T12, $P < 0.05$. 

Fig. 5. Tr and Con groups were tested at B (A), T6 (B), and T12 (C). Values are means ± SE for $n = 20$ subjects (Tr) and $n = 19$ subjects (Con). Acute exercise induces a significant increase in tissue-type plasminogen activator activity (t-PA Act) and antigen (t-PA Ag), t-PA Act/Ag ratio, and urokinase-type plasminogen activator antigen (u-PA Ag) at B, T6, and T12 (not shown). Symbols are defined as in Fig. 3. *Significant difference between Tr and Con in training-induced changes of fibrinolytic parameters (B vs. T6 and B vs. T12), $P < 0.05$. 

![Graphs A, B, C](image)
and recovery could account for cardiovascular events observed after exhaustive exertion (21) in persons with an increased risk profile.

Gris et al. (12) described a decrease in FVII:c after training; however, additional analysis revealed that the reduction in FVII:c was related to the concomitant weight reduction of the participants. In the present study, body composition and dietary habits remained stable during the entire program, and no changes in FVII:c or other coagulation factors (except for FVIII:c) were observed.

Far more attention has been paid to the effect of regular training on overall fibrinolytic activity (11, 27). More recent investigations, dealing with individual components, report higher t-PA Act and lower PAI-1 Ag levels to be responsible for this enhanced fibrinolytic potential (21, 26, 28).

We could not demonstrate a direct effect of training on either t-PA or PAI-1. The (submaximal) training intensity and the (young) age of our subjects may have been important determinants in the outcome of our study. Rankinen et al. (24) trained healthy sedentary men at a comparable submaximal intensity and did not observe training-related changes in basal levels of t-PA or PAI-1. Stratton et al. (26) observed a significant effect of training on t-PA and PAI-1 levels in old male subjects but not in the young participants.

We did, however, observe divergent patterns in PAI-1 and t-PA plasma levels of the Tr and the Con groups that are suggestive of seasonal fluctuations. In the Con group, t-PA Ag as well as PAI-1 Ag and PAI-1 Act showed an unfavorable tendency (29) to increase during the intervention period from February to June, whereas in the Tr group an opposite tendency was observed. This divergent pattern resulted in significant differences among t-PA Ag, t-PA Act/Ag (reflecting fibrinolytic efficacy), and PAI-1 Ag and PAI-1 Act levels of the Tr and Con groups at the end of the intervention period (T12) that could not be attributed to changes in anthropometry (16, 30) or dietary regimen (14).

Comparable seasonal variations in PAI-1 Ag plasma levels of healthy subjects have been observed by Huisveld et al. (16), a finding that has been confirmed by others (7). In patients with rheumatoid arthritis, low PAI-1 levels that cannot be attributed to changes in the carrier protein fibronectin (10) have been observed in early summer (22).

Although higher levels of FVII and Fbg in wintertime that are associated with a higher incidence of cardiovascular diseases have been reported (18, 35), the clinical implications of variations in fibrinolytic components can only be speculated on. The results of the present study suggest that the adverse seasonal effects observed in the Con group are compensated for by the exercise training performed by the Tr group. In addition to body composition (30), dietary habits (15), and physical activity, seasonal variation may influence PAI-1 plasma levels.

The exercise-induced relative (percent) changes in t-PA Ag levels were of a comparable magnitude for both Tr and Con, indicating that not the exercise-induced response but rather the basal plasma levels determine the outcome, as noted before (5). Most likely, PAI-1 is the major fibrinolytic determinant that strongly influences both basal and exercise-induced fibrinolytic activity (13).

Little is known about u-PA in relation to (in)activity. This fibrinolytic activator, like t-PA, demonstrates an increase during exercise, but the regulatory mechanism is vastly different (31).

In both Tr and Con groups, the exercise-induced increase in u-PA was significantly enhanced during intervention. The increase in u-PA Ag was significantly more prominent in the Tr group, suggesting that the training-induced effects were superimposed on the (seasonal) changes that occurred from February to June.

We conclude that regular submaximal physical activity, i.e., training, is associated with an enhanced coagulation (FVII:c) potential and an enhanced fibrinolytic (u-PA) potential. Variations seen in t-PA and PAI-1 in the Con group during the intervention period suggest (unfavorable) seasonal changes that can be reversed by regular physical activity. The observations also stress the importance of the inclusion of Con groups in longitudinal study designs.

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