Strenuous but not moderate exercise increases the thrombotic tendency in healthy sedentary male volunteers

YVES CADROY,1 FABIEN PILLARD,2 KJELL S. SAKARIASSEN,3 CLAIRE THALAMAS,4 BERNARD BONEU,1 AND DANIEL RIVIERE2
1Laboratoire d’Hématologie, Hôpital de Rangueil, 31054 Toulouse Cedex; 2Laboratoire de Physiologie des Adaptations de l’Organisme à l’Exercice Musculaire et des Activités Posturo-Cinétiques, Centre Hospitalier Universitaire Purpan, 31059 Toulouse Cedex, France; 3Division of General Physiology, Department of Biology, University of Oslo, 0317 Oslo, Norway; and 4Centre d’Investigation Clinique, Centre Hospitalier Universitaire Purpan, 31059 Toulouse Cedex, France

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HABITUAL PHYSICAL EXERCISE is associated with an overall decreased risk of acute heart disease. However, intense exercise may trigger acute myocardial infarction (1, 16, 33). Thus a number of studies have shown that strenuous physical exercise resulted in an activation of the hemostatic system (8). This effect depended on the type of physical exercise (24), its duration (21), and its intensity (27). It was different between men and women (29) and between sedentary and trained subjects (26, 30).

Thrombosis is a multifactorial process. The great majority of studies have examined only one aspect of this event, that is platelet function or coagulation or fibrinolysis. The enhanced platelet activation or thrombin generation observed after intense exercise may be opposed by the parallel activation of fibrinolysis (8). In addition, these studies were performed by using in vitro tests, of which clinical relevance is unknown. The overall effect of exercise on thrombogenesis has been rarely investigated in experimental models of thrombosis and has given discordant results (21).

Therefore, we conducted the present study to clarify the effect of both strenuous and moderate acute exercise on arterial thrombogenesis in normal sedentary healthy men with the use of an ex vivo model of arterial thrombosis. In this model, native nonanticoagulated blood is drawn from volunteers through a parallel-plate chamber device where it interacts at well-established flow conditions with collagen, a molecule present in atherosclerotic plaques and primarily responsible for thrombus formation in vivo (27). Blood flow conditions mimic wall shear rates encountered in moderately stenosed (2,600 s⁻¹) small arteries. This model has been used to investigate numerous antithrombotic strategies, and results appear consistent with clinical data (4–7).

SUBJECTS AND METHODS

Subjects. The study population consisted of 15 healthy Caucasian male volunteers aged 20 to 41 yr [25 ± 1 (SE) yr]. These subjects were defined as sedentary because they did not exercise more than two times a week. They had no history or clinical signs of any disease. They were not taking any medication known to affect blood coagulation or platelet function during the 10 days preceding the blood donation. They were nonsmokers or smoked <10 cigarettes/day, and they did not smoke on the day of the perfusion experiments. Their body mass index ranged from 20.7 to 24.8 kg/m² (22.5 ± 0.4 kg/m²). Clinical chemistry, hematologic, and
hemostatic laboratory values were within the normal ranges. All subjects gave written, informed consent to the protocol, which was approved by the local Human Subjects Committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, Toulouse, France).

**Study design.** After selection for the trial, the volunteers were requested to come to the study center to have four sessions of exercise separated by a period of 2–3 wk. The volunteers arrived at the study center at 12:00 PM to receive an adapted meal (~900 kcal; percentage of dietary energy: 15% protein, 30% lipid, and 55% carbohydrate) and to rest for 3 h. Exercise tests began at 3:00 PM. A VO$_2$ max test was performed during the first session to determine the maximum oxygen uptake (VO$_2$ max) and the maximal heart rate. Determinations of VO$_2$ max were performed with an increasing work rate test on a cycle ergometer. The exercise protocol consisted of 2 min of unloaded pedaling followed by a continuous increment of workload, 15–30 W every 3 min. The results showed that their VO$_2$ max was 50.0 ± 1.5 ml·min$^{-1}$·kg$^{-1}$.

The order of the subsequent three sessions was randomized. They were 1) a resting session, 2) a moderate-exercise session in which the exercise was performed during 30 min at a constant workload corresponding to 50% VO$_2$ max, and 3) an intense-exercise session in which the exercise was performed during 30 min at a constant workload corresponding to 70% VO$_2$ max. Blood collection for measurement of platelet aggregation, occlusion time, thrombus formation, and other laboratory tests was performed immediately at the end of each session.

**Preparation of thrombogenic surface.** Equine collagen (Col-lagen Reagent Horn, Nycomed, Munich, Germany) was sprayed onto Thermaxon plastic coverslips (Miles Laboratories, Naperville, IL) to a final density of 0.5 μg/cm$^2$. They were stored at room temperature for 15–20 h before they were used in perfusion experiments (5).

**Perfusion experiments.** Perfusion experiments were performed with a parallel-plate perfusion chamber device at 37°C (5). After blood sample collection, native blood was drawn directly from an antecubital vein through a 19-gauge infusion set (Ohmeda, Helsingborg, Sweden) and over the collagen-coated coverslips positioned in a parallel-plate perfusion chamber. The blood flow rate was maintained at 10 ml/min by a peristaltic roller pump (Minipuls, Gilson, Villiers-Le-Bel, France) placed distal to the chamber. The wall shear rate was 2,600 s$^{-1}$. The time of perfusion was 3 min. The perfusion with blood was followed by a 30-s perfusion of PBS at the same flow rate to wash out blood from the flow channel. The coverslip covered by thrombotic deposits was placed in a plasmakin solution and further processed as described below.

**Immunologic determination of fibrin deposition.** Fibrin deposition was quantified by immunologic determination of fibrin degradation products of plasmkin-digested thrombi as previously described (5). After perfusions, the thrombus was immediately incubated in 2 ml of a plasmkin solution (0.7 IU/ml, in Tris-buffered saline, pH 7.4; Chromogenix, Mölndal, Sweden) for 30 min at 37°C. Fibrin degradation products were measured by using an immunoenzymatic assay (Asserachrom D-Di, Stago, Asnières, France). The amount of desorbed fibrin is directly determined from the levels of fibrin degradation products, which is expressed as fibrin equivalent units according to the manufacturer. Results were expressed as micrograms deposited fibrin per centimeters squared ($\mu$g/cm$^2$).

**Immunologic determination of platelet deposition.** Platelet deposition was quantified by measurement of the specific platelet-gp membrane protein, P-selectin (5). After centrifugation of the plasmkin-digested thrombus, the pellet was dissolved in 400 μl of a lytic buffer, frozen and thawed three times, and then sonicated. The level of P-selectin was measured both in the dissolved pellet and in the supernatant of the plasmkin-digested thrombus by immunoenzymoassay (Bender MedSystems, Vienna, Austria). The total number of platelets deposited was calculated by dividing the amount of P-selectin present in the thrombus by that present in nonactivated platelets of healthy blood donors (321 ± 14 ng/10$^8$ platelets; n = 26). Results were expressed as the number of platelets deposited per centimeters squared ($\times$10$^7$/cm$^2$).

**Other laboratory procedures.** Platelet activation and thrombin generation were determined by measuring plasma levels of β-thromboglobulin (β-TG) and thrombin-antithrombin complexes (T-AT), respectively. β-TG and T-AT were measured in blood (4.5 ml) collected in tubes containing a mixture (0.5 ml) of platelet inhibitors and anticoagulants (sodium citrate, citric acid, theophylline, adenosine, and diprydamole; Diatube, Stago). Blood samples were immediately centrifuged (4,300 g, 19°C, 5 min). The supernatant was collected and centrifuged to eliminate remaining platelets (8,000 g, 19°C, 5 min). Aliquots of plasma were stored at −80°C until assayed. The plasma concentrations of β-TG and T-AT were measured by immunoenzymoassays (Assera-βTG, Stago, and Enzymost-T-AT, Behring, Marburg, Germany, respectively).

For platelet aggregation tests, blood was collected into a citrated vacutainer (Becton Dickinson, Meylan, France) containing 0.5 ml of 0.105 M trisodium citrate for 4.5 ml of blood. Platelet-rich plasma was obtained after a centrifugation at 150 g for 15 min, and platelet-poor plasma was obtained after a second centrifugation at 1,500 g for 15 min. Platelet count was adjusted to 250 × 10$^3$/μl by appropriate dilution of the platelet-rich plasma with autologous platelet-poor plasma. Platelet aggregation was performed with a platelet aggregometer (Helena Laboratories, Beaumont, TX). Agonists were ADP (2.5 and 5 μmol/l final concentrations, Stago) and equine collagen (1 and 5 μg/ml final concentrations, Nycomed). Arachidonic acid-induced platelet aggregation (1 mmol/l final concentration, BioData, Horsham, PA) was also performed to exclude subjects who had taken aspirin. The maximum amplitude of platelet aggregation was measured and expressed as a percentage of the difference between platelet-rich plasma and platelet-poor plasma.

Platelet function was also analyzed with the platelet function analyzer PFA-100, as described by Fressinaud et al. (9). This device uses whole citrated blood, and it measures the time required to obtain the occlusion of a capillary by platelet plug formation under high shear stress with two different agonists, collagen-epinephrine and collagen-ADP. Results are expressed as the closure time (s).

**Von Willebrand factor plasma levels were measured immunologically by using the Laurell method (Assera-vWF, Stago). Fibrinogen plasma levels were measured by the method of von Clauss by the STA automate (Stago). Hematocrit, leukocyte count, and platelet count were measured by an electronic counting device (model S plus, Coulter Electronics, Hialeah, FL).**

**Statistical analysis.** Results were expressed as means ± SE. The data were analyzed by ANOVA with repeated measures for effect of exercise. When ANOVA revealed a significant effect, Fisher’s test was used for post hoc testing to examine the difference between values at baseline and those
obtained after exercise. All statistical tests were two-tailed and were performed at the 0.05 level of significance.

RESULTS

Effect of exercise on physiological and hematologic parameters. The characteristics of exercise testing are shown in Table 1. As expected, exercise increased hematocrit and all blood cell counts in an exercise intensity-dependent manner (P < 0.001; Table 1). Exercise also increased plasma levels of fibrinogen and von Willebrand factor (P < 0.001). The plasma levels of β-TG remained unchanged, but there was an increase in the generation of T-AT complexes, most pronounced at 70% VO₂max (P < 0.01 vs. baseline values).

Effect of exercise on platelet aggregation and hemostatic plug formation. Exercise did not affect platelet aggregation regardless of its intensity when triggered by the agonists ADP or collagen (Table 2). With the use of the PFA-100 analyzer, we found that the closure time induced by collagen-epinephrine and collagen-ADP was shortened by exercise (P < 0.001; Table 2). This shortening was dependent on the intensity of exercise and more pronounced at 70% VO₂max.

Effect of exercise on arterial thrombus formation. Strenuous exercise for 30 min at 70% VO₂max significantly increased platelet thrombus formation (P < 0.01; Table 3). However, fibrin deposition was not affected. Moderate exercise for 30 min at 50% VO₂max affected neither platelet thrombus formation nor fibrin deposition.

DISCUSSION

With the use of a clinically relevant human experimental model of thrombosis, the present study shows that strenuous exercise may increase the risk of arterial thrombogenesis in sedentary young healthy male volunteers. This increased thrombotic tendency was observed only after exercise of heavy intensity (30 min at 70% VO₂max).

Previous studies suggest that the increased thrombotic tendency seen after strenuous exercise may be related to the action of catecholamines. Exercise induces the release of catecholamines (12, 14, 23, 28). At high concentrations, epinephrine promotes platelet aggregation, and at low concentrations, it potentiates platelet aggregation induced by other platelet agonists such as ADP or collagen (11). Thus, in flow conditions typical of atherosclerotic arteries, epinephrine has been found to enhance platelet deposition on severely damaged vessel wall and on collagen fibrils (2, 18). In addition, acute exercise affects the characteristics of the platelet α2-adrenergic receptor by which epinephrine interacts with platelets (10, 13). This effect is intensity dependent (13, 28): whereas moderate exercise does not appear to modify the density and affinity of these receptors, strenuous exercise increases their density on the platelet membrane surface but decreases their affinity for catecholamines. However, the increased thrombotic tendency observed during intense exercise probably includes other factors besides catecholamines (17).

Strenuous exercise also increased thrombin generation (Table 1). In previous studies, an increased thrombin generation with fibrin formation was seen only after prolonged (>30 min) and very heavy exercise.
This increased thrombin generation may be due to the tissue factor activity expression of circulating monocytes, which augments with exercise (15). It may contribute to the exercise-induced thrombotic tendency. However, the increase in thrombin generation, as measured by plasma levels of T-AT in our study, was very modest (Table 1). Also, strenuous exercise had no significant effect on fibrin deposition on collagen (Table 3).

The increased thrombotic tendency may also be related to the observed increased concentration of circulating blood cells and the coagulation factors fibrinogen and von Willebrand factor (Table 1). Indeed, these blood parameters have been shown to markedly influence the occlusion time in the PFA-100 analyzer and platelet thrombus formation occurring in the perfusion chamber system (6, 31). These changes may be due to the hemoconcentration that occurs with exertion, but they also have been shown to persist despite correction for plasma volume changes (25). Exercise-induced release of von Willebrand factor from endothelial Weibel-Palade granules and a reduced clearance of hemostatic factors due to a diminished liver blood flow may also contribute to these findings.

In contrast, the increased thrombotic tendency does not seem to be related to a change in platelet function per se. The susceptibility of platelets to aggregate in response to ADP and collagen did not change (Table 2), and there was no in vivo platelet activation, as indicated by the plasma levels of β-TG (Table 1). Other studies that have examined the effect of exercise on platelet functions have given discordant results, but there were important methodological variations between these studies (8). For example, platelet aggregation is dependent on the platelet count. Therefore, a standardized platelet count is an important parameter to take into account, especially because blood platelet count increases with exercise. Also, the type of exercise (running or bicycle) may influence platelet function (8, 17).

Moderate levels of exercise did not significantly increase the thrombotic tendency (Table 3). Previous studies have already shown that moderate exercise did not increase platelet adhesion and did not promote the release of platelet proteins or in vivo thrombin generation (30, 32). In addition, as previously indicated, moderate exercise does not modify the density and affinity of platelets α2-adrenergic receptors (13).

Criticisms with respect to the significance and clinical relevance of the ex vivo model of human thrombogenesis used may be raised. In our study, thrombus formation was promoted by collagen. Whereas collagen is an important determinant of the thrombogenicity of ruptured human atherosclerotic lesions (27), there are other components that are at least as important, notably tissue factor (24). In addition, we examined the effect of exercise on early acute platelet thrombus formation. Perfusion times were only 3 min because thrombus formation in this model is maximum at 3 min (5). However, one can note that results obtained in this model with widely used antithrombotic agents is consistent with clinical data (5, 7, 20).

The study population consisted of young healthy sedentary volunteers. Different results might have been found if the study had been performed in female (31) or in trained subjects (29). The effect of exercise on thrombosis may also be influenced by the type of exercise. For example, running and treadmill exercise were associated with stronger thrombin generation than found during a bicycle ergometer exertion, probably because the treadmill exercise is associated with a higher degree of tissue damage with enhanced tissue factor-mediated activation of coagulation (17, 19, 25).

In conclusion, the present study shows that strenuous exercise increases the thrombotic tendency, whereas moderate exercise has no such effect. Because reports of arterial thrombosis after vigorous exercise are rare, it is possible that in healthy individuals with an intact endothelium possessing normal antithrombotic properties, vigorous exercise does not present a high risk of thrombosis. However, for patients with coronary artery disease, strenuous exercise may promote a plaque injury and facilitate the formation on this plaque of a platelet-rich thrombus (3). To minimize such a risk, our study suggests that people with coronary artery disease should train predominantly at moderate intensities and avoid heavy exertion.

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REFERENCES


