Moderate-intensity exercise suppresses platelet activation and polymorphonuclear leukocyte interaction with surface-adherent platelets under shear flow in men

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Summary
The reciprocal modulation of platelet and polymorphonuclear leukocyte (PMN) activities is important in the pathogenesis of thrombosis and inflammation. This study investigated how moderate exercise affects shear-induced platelet activation and subsequent PMNs interaction with platelet-related thrombi under shear flow. Sixteen sedentary healthy men engaged in moderate exercise (about 60% VO₂max) on a bicycle ergometer. Platelet activation, PMNs interaction with surface-adherent platelets, and PMN-dependent inhibition of platelet activation under shear flow were measured both before and immediately after exercise. The results of this study can be summarized as follows: (1) moderate exercise was associated with lower extents of shear-induced platelet adhesion and aggregation, binding of von Willbrand factor (vWF) to platelets, and glycoprotein Ibb/IIa activation and P-selectin expression on platelet than at rest; (2) the velocity and percentage of rolling PMNs increased while the number of PMNs remaining bound to surface-adherent platelets decreased after moderate exercise; (3) although treating the PMNs with oxidized-low density lipoprotein (Ox-LDL) enhanced PMNs interaction with surface-adherent platelets, moderate exercise suppressed the enhancement of platelet-PMN interaction by Ox-LDL; (4) moderate exercise decreased platelet [Ca²⁺]i elevation induced by ADP and platelet [Ca²⁺]i levels mediated by PMN and Ox-LDL-treated PMN; and (5) plasma and PMN-derived nitric oxide metabolites and plasma vWF antigen and activity increased after moderate exercise, whereas plasma and platelet-derived soluble P-selectin levels remained unchanged in response to exercise. Therefore, we conclude that moderate-intensity exercise suppresses shear-induced platelet activation and subsequent PMNs adhesion to platelets deposited at sites of vascular injury under flow, thereby reducing the risks of vascular thrombosis and inflammation.

Keywords
Moderate exercise, shear stress, platelet-PMN interaction

Introduction
Regular moderate-intensity exercise training may reduce the risk of major vascular thrombotic events (1, 2) and protect individuals against cardiovascular diseases (3). Enhanced polymorphonuclear leukocyte (PMN)-platelet adhesion has been observed in the circulation of patients with stroke (4) and other vascular diseases (5). Because interaction of platelet and PMN play a key role in thrombus formation and inflammatory response (6), the protective effect of exercise against cardiovascular diseases may partially be due to alterations of platelet and PMN functions.

Platelet activation in pathological vascular microenvironments may trigger PMN stimulation, which can amplify the thrombotic process directly by activating platelets (7, 8). Under certain circumstances, PMN may also exert an antithrombotic
function by inhibiting platelet activation (9). The PMN-derived activity responsible for this inhibition has been identified with nitric oxide (NO), an anti-platelet substance (10). Although acute exercise can increase both platelet and PMN numbers (11-13), the ability of platelet-PMN interaction mediated by acute exercise has been either controversial or incomplete (14-17). These discrepancies may be caused by different methodological determinations, including exercise protocol (i.e., mode, intensity, and duration), and the methods of measuring platelet and PMN functional behaviors in various studies (14-17). The aforementioned studies indicated that moderate-intensity exercise suppressed the adhesiveness of platelets under shear flow conditions (11, 12), while this exercise has a variable influence on PMN function like oxidative burst and phagocytosis (13). Accordingly, we hypothesize that moderate-intensity exercise alters reciprocal modulation of platelet and PMN, possibly affecting the risk of major vascular thrombotic events.

This study elucidates how moderate-intensity exercise affects platelet-PMN interaction under various pathophysiological flow conditions. To specifically assess a multi-step adhesion cascade for PMN interaction with surface-adherent platelets, a linear shear stress flow chamber, that provided a range of shear stresses covering the entire physiological range of human circulation, was used (14). Additionally, shear- and agonist-induced platelet activation and PMN-dependent inhibition of platelet activation were measured to explore the underlying mechanisms of moderate exercise-mediated reciprocal modulation of platelet and PMN activities.

Methods

Subjects

The Ethics Committee of Chang Gung Memorial Hospital reviewed and approved the protocol for this study. The procedures corresponded to institutional guidelines. Sixteen healthy men were studied after they had given their informed consent and the experimental procedures were explained to them. The physical characteristics of these subjects, expressed as mean ± SEM, were age 22.1±0.6 years; height, 171.3±1.7cm; and body weight, 73.5±2.1kg. None of the subjects had engaged in any regular physical activity for at least 1 year before the study. All subjects were nonsmokers, and abstained from all medication at least for 2 weeks before the study. On the day of the study, the subjects had a light mixed breakfast without any drinks containing caffeine at 8:00 AM, and then fasted for 5 hours prior to participating in the study. All subjects arrived at the testing center at 1:00 PM to avoid a possible diurnal influence.

Exercise and blood collection protocol

At the beginning, a progressive exercise test was performed in each subject. The exercise protocol for these subjects comprised 2 min of unloaded pedaling, after which the workload was increased incrementally by 20 to 30 Watt every 3 min until exhaustion. This exercise test was used to determine maximal oxygen consumption (VO_{2max}) in tested subjects, as described elsewhere (11), and the value of their VO_{2max} was 31.8±2.5 mL/min/kg (mean±SEM). To avoid the accumulated effects of repeated acute exercise, all subjects performed the second exercise protocol (i.e., moderate-intensity exercise) 5 days after a progressive exercise test. At this time, the subjects had arrived at the laboratory and rested for 30 min, blood samples were drawn from a forearm vein. The first 2 mL of blood was discarded, and the remaining blood sample was then used to measure resting hematological parameters and blood cell functions. Moderated-intensity exercise protocol was performed approximately 60% (i.e., 17.8±1.3 mL/min/kg; 60.6±1.9%) of predetermined VO_{2max} for 40 min. Immediately after this exercise, another blood sample was collected to measured post-exercise hematological parameters and blood cell functions.

Shear-induced platelet aggregation and adhesion

Ten mL blood samples were transferred into polypropylene tubes containing sodium citrate (3.8 g/dL: 1 vol. to 9 vol. of blood) (Sigma Chemical Co., St Louis, Mo, USA). Platelet-rich plasma (PRP) was prepared by centrifugation at 120g for 10 min at room temperature, while platelet-poor plasma (PPP) was obtained by recentrifugation at 1600g for 10 min, also at room temperature. The number of platelets in PPP was adjusted to 2 × 10^8 cells/mL. Sixty μL PRP was placed in a 1 mg/mL albumin (Sigma Chemical Co., St Louis, Mo, USA) or 1 mg/mL fibronectin (Sigma Chemical Co., St Louis, Mo, USA)-coated glass plate (32 mm diameter) and sheared at controlled levels of shear stress at 37°C for 5 min using a rotational viscometer (CAP2000; Brookfield, Middleboro, MA, USA). The rotational viscometer comprised a stationary plate and a silicon-coated rotating cone that imposed a uniform shearing motion on the entire sample. The diameter of the cone was 3 cm and its angle was 0.45°. The viscosity of the PRP was estimated to be 1cp. The PRP samples were exposed to high shear stress (200 dyne/cm^2 for 5 min; rotating speed of cone = 1500 rpm) and low shear stress (10 dyne/cm^2 for 5 min; rotating speed of cone = 75 rpm) at 37°C. In PRP on albumin-coated well, the PRP suspension was removed from the well immediately following exposure to shear stress, to count the single platelets. The measured numbers of aggregated platelets were expressed as percentages of total number of platelets, (single platelet count before shear stress – single platelet count after shear stress)/ single platelet count before shear stress X 100% (11). In PRP on fibronectin-coated well, platelets adhering on the wells were then fixed with freshly prepared glutaraldehyde (0.25% final concentration), and the surface coverage of adhered platelets per unit area (0.16 mm^2) was analyzed with an inverted light microscope.
(Nikon Co, Tokyo, Japan) connected to an image analysis system (Motic Images plus 2.0, Micro-optic Industrial Group Co. Ltd., Richmond, Canada) (14).

**Ristocetin-induced platelet aggregation**
Platelets were washed by repeated centrifugation with an albumin cushion, as described elsewhere (18). The aggregation of platelets was induced by ristocetin (Sigma Chemical Co., St Louis, Mo, USA) in the presence of 10 µg/mL vWF (Calbiochem, San Diego, CA, USA) at varying concentrations (0.25, 0.5, 0.75, 1 mg/mL) of ristocetin (Sigma Chemical Co., St Louis, Mo, USA). Results were expressed as the percentage of aggregated platelets to total number of platelets (11).

**Binding of vWF to platelets**
Washed platelet suspensions (50,000 platelets/µL) with 10 µg/mL vWF (Calbiochem, San Diego, CA, USA) were exposed to varying shear stresses, as described previously (19). Aliquots (20 µL) of washed platelets were fixed immediately following shearing using an equal volume of 2% formaldehyde in phosphate-buffered saline (PBS) (Sigma Chemical Co., St Louis, Mo, USA). The aliquots were incubated with saturating concentrations of rabbit anti-human vWF IgG (Sigma) and FITC-conjugated anti-rabbit IgG (Sigma Chemical Co., St Louis, Mo, USA) (20 µg/mL) for 30 min in the dark. Samples were diluted with 1% formaldehyde, and analyzed in a FACScan flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA). Control (non-sheared) samples were introduced into the viscometer, but were not exposed to shear stress. A gate was set around single platelets. Ten thousand events were acquired and analyzed for FITC fluorescence, considered as an index of binding of the reporter anti-human FITC antibody. Results were expressed as the percentage of vWF-positive platelets above the threshold fluorescence of the control platelet samples (19).

**Activation of glycoprotein (GP) IIb/IIIa and expression of P-selectin on platelets**
Washed platelet suspensions with vWF were exposed to varying shear stresses, and aliquots were then incubated with a saturating concentration of FITC-conjugated PAC-1 (activated form of GP IIb/IIIa) (Pharmingen, San Diego, CA, USA), FITC-conjugated anti-CD62P (P-selectin) (Serotec Inc. Raleigh, NC, USA), or FITC-conjugated anti-rabbit IgG control antibodies (Serotec Inc. Raleigh, NC, USA) for 30 min in the dark. After fixation with 1% formaldehyde in PBS, the fluorescence obtained from 10,000 events that represented platelets, was determined using a flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA). Parallel, non-sheared samples were used to correct for background fluorescence. In sheared samples, the increase in fluorescence resulting from anti-CD62P antibody and PAC-1 beyond this background was expressed as a percentage of the 10,000 counted events.

**PMNs interaction with surface-adherent platelets under flow**
Peripheral blood PMNs were isolated from whole venous blood by dextran sedimentation followed by density separation over Ficoll-Hypaque and hypotonic lysis (20). The PMNs were then resuspended in Tyrode-N-2-hydroxyethylpiperazine -N'2-ethanesulfonic acid (Tyrode's-HEPES) buffer, and the PMNs were adjusted to 1 × 10⁷ cells/mL. For certain experiments, buffer or 100 µg/mL oxidative-low density lipoprotein (Ox-LDL) was added to the washed PMNs suspension, which was then incubated at 37°C for 1 hour. Ox-LDL was prepared from fresh human plasma, as described in our previous study (21). After incubation, PMNs were washed and resuspended with Tyrode's-HEPES (1 × 10⁷ cells/mL). All platelet and PMN samples were used within 2 hours of purification.

A tapered parallel plate chamber, which provided shear stress values covering the entire physiological range of human circulation, was used to assess a multi-step adhesion cascade for PMNs interaction with surface-adherent platelets, as described previously (14).

**Platelet and PMN-mediated platelet [Ca²⁺]ᵢ**
Platelets were washed by repeated centrifugation with an albumin cushion and labeled using a calcium-sensitive fluorescent dye, fura-2 AM (Sigma Chemical Co., St Louis, Mo, USA), as described previously (18). Some PMNs were fixed with 0.25% glutaraldehyde (J.T. Baker Co., Phillipsburg, NJ, USA) for 5 mins and washed three times with buffer prior to use. Fixed, unfixed, and Ox-LDL-treated PMNs were preincubated with 100 U/mL superoxide dismutase (for inhibiting oxidation of PMN-derived NO) (Sigma Chemical Co., St Louis, Mo, USA) and 1 µM fMLP (Sigma Chemical Co., St Louis, Mo, USA) for 1 minute prior to coincubation with fura-2 loaded-platelets, and these were cells then mixed (2 × 10⁷ PMNs/mL plus 4 × 10⁸ platelet/mL) in Tyrode-HEPES buffer. Finally, 10 µM ADP-evoked platelet [Ca²⁺], levels were calculated according to the ratio values of fluorescence intensities measured at excitation wavelength of 340 nm and 380 nm in an intracellular calcium measurement instrument (Caf 110, Jasco Corp., Tokyo, Japan).

**Plasma and PMN-derived NO metabolites**
Plasma and cell-derived nitrate plus nitrite levels were measured by the Griess reagent-based colorimetric method (22), using commercially available assay kit (Cayman Chemical Co., Ann Arbor, MI, USA), as described before (14).

**vWF antigen and activity and soluble P-selectin (sP-selectin)**
Plasma vWF antigen (Corgenix, Westminster, CO, USA) and activity (American Biochemical and Pharmaceutical Co., New York, USA) as well as plasma and platelet-derived sP-selectin
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Contents were measured using commercial ELISA, as described before (14).

Statistical analysis
The Statview IV statistical software package was used for data analysis. All data were expressed as mean ± SEM. The effects of moderate exercise on changes in hematological parameters were compared using the paired Student’s t test. To compare the differences in blood cell functions under various experimental conditions, at rest and immediately after exercise, the results were analyzed by repeated measure ANOVA and Tukey’s multiple range test. Differences were considered significant at P<0.05.

Results

Immediately after moderate exercise, the subjects displayed increased levels of the hematological parameters, i.e., RBC, WBC (including PMN, Lym, and Mon of its subgroups), Plt, Hct, and Hb (Table 1). Although plasma and platelet-derived sP-selectin contents did not change significantly after moderate exercise, plasma and PMN-derived NO metabolites and vWF antigen and activity increased after exercise (Table 2). PMNs appeared to decrease platelet-derived sP-selectin content. However, no significant changes occurred in the PMN-dependent inhibition of platelet-derived sP-selectin following exercise (Table 2).

Measured results revealed that a high shear stress induced a higher platelet adhesion, indicated by a larger percentage of surface coverage (Fig. 1A), and aggregation (Fig. 1B) as well as the bindings of vWF and PAC-1 to platelets (Figs. 2A and 2B) and the expression of P-selectin on platelet (Fig. 2C) than a low shear stress. However, moderate exercise reduced shear-induced platelet adhesion (Fig. 1A; P<0.001) and aggregation (Figs. 1A and B; P = 0.008), which was accompanied by a decrease in vWF binding (Fig. 2A; P = 0.007), GP IIb/IIIa activation, indicated by a lower PAC-1 bound (Fig. 2B; P = 0.002), P-selectin expression on platelet (Fig. 2C; P = 0.017) under high shear stress. Figure 1C illustrates the dose responses of platelet aggregation induced by ristocetin at rest and immediately after moderate exercise. Moreover, the platelet aggregation induced by 0.75 mg/mL of ristocetin was reduced by moderate exercise (Fig. 1C, P < 0.001).

Moderate exercise increased the mean velocity and percentage of rolling PMNs (Figs. 3A and 3B, P = 0.012 and P = 0.011, respectively) and decreased the number of PMNs remaining bound to surface-adherent platelets (Fig. 3C; P = 0.019) under

Table 1: Comparison of blood cell counts at rest and immediately after moderate exercise.

<table>
<thead>
<tr>
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<th>R</th>
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<tr>
<td>RBC, mm³</td>
<td>4.81±0.08</td>
<td>5.03±0.07*</td>
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<tr>
<td>Hct, %</td>
<td>43.0±0.9</td>
<td>44.7±0.6*</td>
</tr>
<tr>
<td>Hb, g/mL</td>
<td>13.6±0.4</td>
<td>14.2±0.4*</td>
</tr>
<tr>
<td>WBC, mm³</td>
<td>6.32±0.36</td>
<td>8.21±0.53*</td>
</tr>
<tr>
<td>PMN, mm³</td>
<td>4.30±0.37</td>
<td>5.40±0.54*</td>
</tr>
<tr>
<td>Lym, mm³</td>
<td>1.93±0.08</td>
<td>2.60±0.18*</td>
</tr>
<tr>
<td>Mon, mm³</td>
<td>0.13±0.01</td>
<td>0.17±0.02*</td>
</tr>
<tr>
<td>Plt, mm³</td>
<td>202±13</td>
<td>235±15*</td>
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Values are mean ± SEM. * P<0.05, R vs. E

Table 2: Comparison of vWF antigen and activity, NO metabolite levels, and soluble P-selectin contents at rest and immediately after moderate exercise.

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<thead>
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<tr>
<td>Plasma vWF</td>
<td></td>
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<tr>
<td>Antigen, IU/dL</td>
<td>87.6±12.3</td>
<td>112.7±15.3*</td>
</tr>
<tr>
<td>Activity, IU/dL</td>
<td>78.7±9.5</td>
<td>94.3±12.8*</td>
</tr>
<tr>
<td>Nitrite plus Nitrate</td>
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<tr>
<td>Plasma, µM</td>
<td>29.2±3.9</td>
<td>62.5±11.8*</td>
</tr>
<tr>
<td>PMN, nmole/10⁷ PMNs</td>
<td>13.7±0.6</td>
<td>17.7±1.0*</td>
</tr>
<tr>
<td>PMN + Plt, nmole/10⁷ PMNs+2x10⁵ Plts</td>
<td>13.6±0.4</td>
<td>16.9±0.6*</td>
</tr>
<tr>
<td>Soluble P-selectin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma, ng/mL</td>
<td>31.8±3.1</td>
<td>30.6±3.1</td>
</tr>
<tr>
<td>Plt, ng/2x10⁸ Plts</td>
<td>11.8±0.4</td>
<td>13.7±1.9</td>
</tr>
<tr>
<td>PMN + Plt, nmole/10⁷ PMNs+2x10⁵ Plts</td>
<td>7.9±1.8*</td>
<td>7.2±0.6*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Plt = platelet, * P<0.05, R vs. E.
shear stress from 4 to 28 dynes/cm². For shear stress of below 4 dynes/cm², the platelet-PMN interaction remained unchanged in response to moderate exercises (Fig. 3). In treating the PMNs with Ox-LDL, a decrease in the mean velocity and percentage of rolling PMNs was observed, along with an increase in the number of PMN remaining bound to the platelet surface under various shear stresses (Figs. 3D-3F). However, the reduced rolling (P = 0.020) and the enhanced adhesion (P = 0.015) of PMNs by Ox-LDL were suppressed by moderate exercise.

Although PMNs co-incubated with platelets (Plt + PMN) reduced ADP-induced platelet [Ca²⁺]ᵢ rise, treating the PMNs with Ox-LDL (Plt + Ox-LDL-PMN) decreased the extent to which platelet [Ca²⁺]ᵢ rise was depressed by PMN (Fig. 4). ADP-induced platelet [Ca²⁺]ᵢ rise under fixed, unfixed, and Ox-LDL-treated PMNs co-incubated with platelets after moderate exercise was lower than that at rest (Fig. 4; P<0.001). However, no significant changes occurred in basal platelet [Ca²⁺]ᵢ level under all experimental conditions following this exercise (data no show).

Discussion

Lifestyle habits such as exercise may have significant influence on cardiovascular disease (23). Previous studies have suggested that the risk of primary cardiac arrest is transiently increased during vigorous exercise, whereas moderate-intensity exercise training is associated with an overall decreased risk of primary cardiac arrest (24). The hemostatic system plays an important role in the pathogenesis of cardiovascular disease (23). Our previous studies indicated that the intensity of acute exercise is an important factor affecting blood platelet function; i.e., moderate exercise tends to desensitize platelets, whereas strenuous exercise can potentiate platelets either in healthy subjects (11, 12), or in patients with stable angina (11). Moreover, blood coagulation and fibrinolysis are also affected by acute exercise in an intensity-dependent manner; i.e., moderate exercise appears to enhance in vivo fibrinolysis, whereas very heavy exercise activates blood fibrinolysis and coagulation simultaneously (25). On the other hand, platelet and PMN co-localization to
the wall of damaged or stimulated blood vessel is an essential component of a multistep cascade in thrombosis and inflammation (20). To our knowledge, this investigation is the first to clearly demonstrate that moderate, acute exercise decreases shear-induced platelet activation, and subsequent PMN interaction with surface-adherent platelets under flow, thereby reducing the risks of vascular thrombosis and inflammatory response.
Platelet activation induced by shear stress occurring in a stenosed artery is one of the mechanisms of arterial thrombogenesis (26). Pathological, high shear stress induces the binding of vWF to the GP Ib complex on platelets. This interaction transduces signals in platelets, subsequently activating GP IIb/IIIa. The activated GP IIb/IIIa complex then binds to fibrinogen which stabilizes aggregated platelets (27, 28). The study demonstrates that although moderate exercise modestly increases plasma vWF levels, there are significant decreases in shear-induced platelet adhesion on fibronectin-coated surface and vWF binding to platelets after this exercise. Fibronectin is present in plasma, the subendothelium of the vessel wall, an in the α granules of platelet, and it supports platelet adhesion and spreading under static or flow conditions (29, 30). A previous study has suggested that GP Ib is a mediator of platelet adhesion to fibronectin under flow, and that GP Ib-mediated adhesion to fibronectin depends on plasma or platelet vWF (30). Therefore, it seems that moderate exercise appears to somehow influence the performance of platelet fibronectin receptors or/and GPIIb-vWF-mediated platelet activation. Ristocetin, a vancomycin-like antibiotic from Nocardialaurida, can induce binding of the vWF A1 domain to the GP Ib complex on a platelet (31). Therefore, ristocetin was used to indicate the capacity of vWF to bind to GP Ib complex in this study. A previous study displayed that platelet aggregation was induced by ristocetin from 0.7 to 1 mg/mL in healthy subjects (32). The results obtained here reveal that moderate exercise decreased platelet aggregation induced by 0.75 mg/mL ristocetin, implying that this exercise suppresses the capacity of vWF to bind to GP Ib complex on platelet.

The binding of vWF to platelet GP Ib complex under a high shear stress has previously been demonstrated to cause intracellular Ca²⁺ signaling and GP Ib/IIa-dependent platelet aggregation (33). When [Ca²⁺], increases in a platelet, both P-selectin and ADP can be released from the α-granules in the platelet (34), subsequently inducing the expression of P-selectin and the activation of GP IIb/IIIa on the platelet membrane. The results of this study show that a high shear stress in the presence of exogenous vWF evoked both the activation of GP IIb/IIIa and the expression of P-selectin on platelets. However, moderate exercise decreased the extent of shear-induced GP IIb/IIIa activation and P-selectin expression on platelets.

The suppressed platelet activity in moderate exercise noted in this study may attenuate the formation of hemostatic platelet plugsing, which may in turn reduce the risk of vascular thrombosis. Interestingly, the measured results of this study displayed that moderate exercise also significantly increased the velocity and number of rolling PMNs and reduced the number of PMN remaining bound to surface-adherent platelets under shear flow. This finding implies that moderate exercise also can effectively suppress co-localization of PMN and platelet to the wall of damaged or stimulated blood vessels, subsequently inhibiting inflammatory responses. However, some studies found that platelet-leukocyte conjugates increased after short-term strenuous exercise (15, 16) or long-term submaximal exercise (i.e., 90% individual anaerobic threshold) (17), contradicting the observations of this investigation. This investigation used moderate, acute exercise and specifically designed a physiological shear flow condition to observe PMN interaction with surface-adherent platelets, whereas other studies (15-17) used vigorous or endurance exercise and lacked the shear flow control. Therefore, the effects of acute exercise on platelet-PMN interaction may be related to different exercise protocols and different techniques for the evaluation of platelet and PMN function.

P-selectin expression on the surface of immobilized, activated platelets supports tethering and rolling of PMNs from the blood stream (20). Subsequent firm adhesion and transmigration across adherent platelets in a chemotactic gradient is mediated through interaction of GP IIb/IIIa and Mac-1 (20, 35). Therefore, moderate exercise-induced change in platelet-PMN interaction may be due to reduced platelet GP IIb/IIIa activation and P-selectin expression, since these adhesion molecules of platelets are involved in the adhesion of activated platelets to PMNs. The underlying mechanisms by which moderate exercise influences the expressions of membrane-associated adhesion molecules on platelets remain unclear. A previous study demonstrated that moderate exercise induces a substantial release of NO, probably from the vascular endothelium, increasing platelet cGMP and desensitizing platelets (12). NO inhibits the formation of thrombus under high shear flow (36) and attenuates agonist-induced upregulation of P-selectin and the GPIIb/IIIa by negatively regulating the cGMP in platelets (37). By increasing NO release, moderate exercise may decrease the performance of adhesion molecules on platelets, thereby reducing the platelet activation induced by shear stress and suppressing PMN interaction with surface-adherent platelets under flow.

Physical exercise causes significant oxygen inhalation, and earlier studies have proven that the blood is subjected to oxidative stress during exercise (38). However, small amounts of oxygen-derived free radicals are normal, scavenged by endogenous antioxidant, such as NO (39). A previous study (14) and this investigation indicated that treating the PMNs with Ox-LDL (as a stimulator of oxidative stress) can facilitate PMNs adhesion to platelet-rated thrombi and attenuate PMN-dependent antiplatelet function. According to our present results, moderate exercise significantly inhibits the oxidative stress effects, and is accompanied by an increase in plasma and PMN-derived NO levels. Therefore, although moderate exercise tends to be associated with higher oxidative stress, because of the inhalation of more oxygen, than at rest, the enhanced PMN-derived antioxidants in moderate exercise, such as NO, may suppress oxidative stress-promoted interaction between platelets and PMNs.
In conclusion, moderate-intensity exercise may directly suppress platelet activation induced by physical shear forces and chemical agonists. Moreover, the exercise also suppresses PMN interaction with surface-adherent platelets under shear flow. Therefore, moderate-intensity exercise can plausibly be considered a “safe” level of exercise for minimizing vascular complication by eliciting beneficial physiological changes.

Acknowledgments
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References