High Intensity Exercise Enhances Platelet Reactivity to Shear Stress and Coagulation during and after Exercise

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Key Words

Platelet reactivity · Coagulation · Shear forces · Exercise intensity · Ventilatory threshold

Abstract

Platelets play a crucial role in the pathogenesis of acute cardiac events, such as angina, myocardial infarction and sudden death. It is believed that regular low-intensity exercise can reduce, while high-intensity exercise may provoke acute cardiac events. The aim of the present study was to investigate the effect of acute exercise both at low and high intensities on the ventilatory threshold (VT), platelet reactivity and coagulation before and after exercise. Platelet reactivity and coagulation were measured under flow condition, using native blood, by hemostatology. Seven healthy young men (age: 20–29 years) performed bicycle ergometer exercise for 30 min at intensities of 90% (Ex-VT90% or approximately 55% $\text{VO}_{2\text{max}}$) and 130% (Ex-VT130% or 80% $\text{VO}_{2\text{max}}$) of individual VT. Blood cell counts, hematocrit, blood lactic acid and plasma catecholamine levels were slightly but significantly increased after Ex-VT90% and markedly after Ex-VT130% after 30 min exercise. Subsequent to the exercise, the elevated blood cell counts decreased to the

resting levels both at Ex-VT90% and at Ex-VT130%. Platelet reactivity to shear stress and dynamic coagulation were significantly enhanced immediately and 30 min after Ex-130%VT. In contrast, no significant changes occurred in those of Ex-90%VT. The present study suggests that high-intensity exercise-induced platelet hyperreactivity and hypercoagulable state may pose an increased risk for acute, sometimes fatal cardiac event. On the other hand, our findings support the view that low-intensity exercise does not present a risk of thrombosis.

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Introduction

There is ample evidence for the beneficial, preventive effect of exercise in the so-called 'life-style related diseases'. However, there is also a 'paradox of exercise' [1, 2], which means that exercise may precipitate acute cardiac events and sudden cardiac death. Platelets and coagulation play a pivotal role in building up an occlusive platelet-rich thrombus in an affected coronary artery [3]. The effect of exercise on thrombus formation has been studied extensively, but findings are inconsistent and controver-

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sial [4, 5]. This may be due to the methods employed for measuring platelet function or overall thrombotic status. Opposed to the battery of chemical agonists employed by the conventional platelet function tests to induce platelet aggregation, there is convincing evidence that under pathological conditions such as rupture of an atherosclerotic plaque or luminal stenosis, it is the shear stress and flow disturbances which mainly account for the development of an arterial thrombus [6–8]. Despite this, studies of the effect of acute exercise of different intensities on platelet function and coagulation are rarely performed under flow condition [9, 10].

In our earlier studies on the effect of exercise, we used shear-induced in vitro platelet reactivity tests, such as the Thrombotic Status Analyzer (TSA) and Hemostatometer. With these techniques, we have shown immediate enhancement of platelet reactivity after exercise at moderate intensity and the involvement of an increased blood level of norepinephrine in such platelet effect [11–13]. These studies suggested that it was the intensity of exercise, rather than its duration, which determined the biological effect. However, high-intensity exercise has not been investigated with these techniques. Further, exercise presents a risk for acute cardiac events not only immediately after exercise, but also later, during the ‘recovery period’ [14]. The effect of exercise of different intensities on shear-induced platelet reaction and coagulation during and especially after exercise, in the recovery period, has not yet been investigated. The aim of the present study was to investigate the effect of exercise at low and high intensities based on ventilatory threshold (VT), platelet reactivity and coagulation during, immediately after exercise and in the recovery period.

Materials and Methods

Subjects

Seven healthy male volunteers who regularly took moderate exercise were studied. Mean age was 21.9 ± 1.2 years (mean $\pm$ SEM), height 175.1 ± 1.7 cm and body weight 64.0 ± 2.1 kg. None of the subjects was a smoker or has taken any medicine for 2 weeks before the measurement. Prior to the experiment, the aim, details, and procedure were explained to the subjects and informed consent was obtained.

Experimental Procedures

To determine individual VT, the subjects first performed on a bicycle ergometer (Aerobike 820, Combi, Japan) with stepwise increases in intensities (after 3 min at 30 W, with 15-watt increases every 1 min until exhaustion). Maximal oxygen uptake ($\text{VO}_{2\text{max}}$) was also measured during exercise and individual $\text{VO}_{2\text{max}}$ was 49.7 ± 2.9 ml/kg/min (mean $\pm$ SEM). Individual VT was measured using

computerized regression analysis of the slopes of CO_2 production, O_2 uptake (VO_2), and minute ventilation plot (model AE-280S, Minato, Japan), called the ‘V-slope method’ [15, 16].

Using a bicycle ergometer, each subject exercised for 30 min at intensities of 90% (Ex-90%VT) and 130% (Ex-130%VT) of the individual VT. Ex-90%VT and Ex-130%VT corresponded to $55.6 \pm 1.6\%$ (mean $\pm$ SEM) and $80.1 \pm 2.2\%$ $\text{VO}_{2\text{max}}$, respectively. We monitored the heart rate of each subject during exercise and controlled the work rate in such a way that the heart rate corresponded to 90%VT or 130%VT. The exercise was performed at a room temperature of $23 \pm 0.5^\circ\text{C}$, at the same time of the day on two separate days.

Blood samples were collected from the antecubital vein before (at rest), immediately after and 30 min after exercise. The first 7-ml blood sample was used to measure blood lactic acid levels, blood cell counts, hematocrit, and plasma catecholamine levels. The second 7-ml blood sample was used for hemostatometry.

Subjects were asked not to take any food or drink at least 2 h before the beginning of the exercise.

Measurement of Thrombotic Status (Hemostatometry)

The Hemostatometer, as described by Kovacs et al. [17] was custom-built in the workshop of the Laboratory of Physiology, Faculty of Nutrition, Kobe Gakuin University. The principle of the instrument is shown in figure 1. Two milliliters of nonanticoagulated blood was drawn into a preheated syringe and the syringe was inserted into a heated (37°C) metal block (fig. 1A). Liquid paraffin was pumped into the blood at a constant speed, thus displacing blood from the syringe into a polyethylene tube (fig. 1B), which ended in a reservoir, filled with paraffin oil (fig. 1C). Two minutes after the blood started to flow into the plastic tubing, the latter was pierced with a fine needle making two holes in the wall of the tubing. The blood escaping through the holes simulated ‘bleeding’. The initial shear stress in the holes was 625 dyne/cm². Due to platelet-rich hemostatic plug formation in the holes, ‘bleeding’ stops, thus simulating hemostasis. After hemostasis, blood continues to flow into the plastic tube until coagulation occurs, which permanently arrests the flow. The perfusion pressure was monitored to analyze the different events. The characteristic pressure changes due to piercing the tube, during hemostasis and subsequent coagulation are shown in figure 2. The drop of perfusion pressure from the prepunch level to the lowest pressure level was taken as 100%. Area (mm Hg $\times$ s) under the recording of 30% recovery of pressure (H1, initial platelet reaction) and 90% recovery of pressure (H2, overall hemostasis) were calculated and used as indices of platelet reactivity. The time from the start of the test until the first (>10 mm Hg) pressure drop subsequent to hemostasis (CT1, beginning of coagulation, min) and until the pressure drop to ≤ 10 mm Hg (CT2, dynamic clotting time) were used as indices of coagulation. A three-channel instrument was used, which allowed three parallel measurements to be performed in the same time.

Other Measurements

From the first drawn 7-ml blood sample, blood cell counts and hematocrit were measured with an automated blood cell counter (Microcellcounter SF-3000, Sysmex, Japan). The rest of the blood was anticoagulated with EDTA, centrifuged at 3,000 rpm for 15 min at 4°C , and the plasma separated. Plasma catecholamines (epinephrine and norepinephrine) were measured by high-performance liquid chromatography; lactic acid concentration was measured with a lactate test meter (Lactate ProTM LT-1710, Arkray Factory Inc.,

Fig. 1. Scheme of the Hemostatometer. **A** Native blood in a syringe is kept vertically in a holder at 37°C. Native blood is displaced by liquid paraffin at 0.05 ml/min resulting in blood flow in polyethylene tubing (0.50 mm i.d., 1.00 mm o.d.). **B** Bleeding into the surrounding saline at 37°C is caused by puncture with a needle (0.18 mm in diameter), followed by hemostasis due to shear-induced platelet-rich plug formation. **C** Polyethylene tubing is connected to a blood waste reservoir with a pressure under 60 mm Hg and changes in pressure are recorded.

Fig. 2. A typical hemostatogram. ▼: piercing; H1 (▨): the area until 30% pressure recovery; H2 (▩): the area until 90% recovery; CT1: the time until a pressure drop of 10 mm Hg from the baseline (60 mm Hg) caused by clotting; CT2: the time until drop to 10 mm Hg.

Japan). Body weight was measured with a digital body weight scale (TBF-511, Tanita, Japan) and heart rate with chest bipolar leads.

Statistical Analysis

Hemostatometry measurements were performed in triplicate by using three channels of the instrument, and from the three parallels mean values were calculated. The results are shown as means $\pm$ SEM. Platelet reactivity indices (H1; H2) were converted to logarithms before statistical analysis, whilst the raw data of coagulation

indices (CT1; CT2) were analyzed, as in our previous papers [7, 12]. Results were analyzed for each exercise test by one-way repeated-measures ANOVA, followed by Fisher's PLSD as post-hoc test to examine the difference between values at rest (before exercise) and those obtained immediately or 30 min after exercise. Analysis was performed using the commercially available statistical package Stat View (version 5.0; SAS Institute Inc., USA). A p value <0.05 was considered to be statistically significant.

Table 1. Effects of Ex-VT130% and Ex-VT90% on heart rates, body weight, and blood markers

		Before	Immediately after	Recovery 30 min	p value (ANOVA)
Heart rate, beats/min	Ex-VT130%	64 ± 2	173 ± 3***	65 ± 1	<0.001
	Ex-VT90%	65 ± 1	142 ± 3***	64 ± 1	<0.001
Body weight, kg	Ex-VT130%	64.6 ± 2.0	63.9 ± 2.0***	63.8 ± 2.0***	<0.001
	Ex-VT90%	64.4 ± 2.1	64.0 ± 2.1***	63.9 ± 2.1***	<0.001
Erythrocytes, × 10 ⁴ /μl	Ex-VT130%	513 ± 9	549 ± 8***	506 ± 8	<0.001
	Ex-VT90%	512 ± 13	539 ± 13***	510 ± 13	<0.001
Leukocytes, × 10 ² /μl	Ex-VT130%	53.4 ± 2.8	86.1 ± 5.4***	53.6 ± 4.0	<0.001
	Ex-VT90%	53.1 ± 3.8	67.6 ± 5.4***	49.2 ± 4.4	<0.001
Platelets, × 10 ⁴ /μl	Ex-VT130%	21.4 ± 1.6	26.1 ± 2.3***	21.3 ± 1.7	<0.001
	Ex-VT90%	20.7 ± 2.4	23.3 ± 2.6**	20.4 ± 2.4	<0.001
Hematocrit, %	Ex-VT130%	46.6 ± 0.9	49.9 ± 0.9***	45.8 ± 0.9	<0.001
	Ex-VT90%	46.5 ± 1.3	49.0 ± 1.2***	46.3 ± 1.3	<0.001
Lactic acid, mM	Ex-VT130%	0.8 ± 0.1	5.5 ± 1.3***	1.5 ± 0.3	<0.001
	Ex-VT90%	0.7 ± 0.003	1.5 ± 0.3*	0.8 ± 0.1	0.020
Epinephrine, nM	Ex-VT130%	0.434 ± 0.057	1.510 ± 0.402**	0.480 ± 0.051	<0.001
	Ex-VT90%	0.323 ± 0.039	0.625 ± 0.102***	0.263 ± 0.047	<0.001
Norepinephrine, nM	Ex-VT130%	4.046 ± 0.473	13.679 ± 1.021***	4.396 ± 0.324	<0.001
	Ex-VT90%	3.985 ± 0.355	7.112 ± 0.584***	3.941 ± 0.455	<0.001

Values are expressed as the mean ± SEM. * p < 0.05, ** p < 0.01, *** p < 0.001 compared with before exercise (Fisher's PLSD).

Results

Effects of Exercise on Heart Rate and Body Weight

The effects of Ex-130%VT and Ex-90%VT on heart rate and body weight are shown in table 1. The average heart rate immediately after exercise Ex-VT90% was 142.0 ± 2.9 beats/min, and at Ex-130%VT was 173.3 ± 3.2 beats/min. These heart rates changed very little during the exercise. Thirty minutes after the exercise, heart rates decreased to the levels measured at rest. Body weight significantly decreased immediately after exercise (p < 0.001 for both Ex-130%VT and Ex-90%VT), and remained low 30 min after exercise (p < 0.001 for both Ex-130%VT and Ex-90%VT).

Effects of Exercise on Blood Cell Counts, Hematocrit, Blood Lactic Acid Levels, and Plasma Catecholamine Concentrations

Table 1 shows the effects of Ex-130%VT and Ex-90%VT on blood cell counts, hematocrit, blood lactic acid levels, and plasma catecholamine concentrations. Immediately after exercise, blood cell counts, haematocrit, blood lactic acid levels, and plasma catecholamine levels increased slightly (but statistically significantly) at Ex-

VT90% and remarkably at Ex-VT130%. Thirty minutes after the exercise at both intensities, these values returned to the levels measured before the exercise.

Effects of Exercise on Platelet Reactivity and Coagulation

The effects of Ex-130%VT and Ex-90%VT on platelet reactivity (H1 and H2) and coagulation (CT1 and CT2) are shown in figures 3, 4. Ex-130%VT increased platelet reactivity both immediately after exercise (p < 0.05 for both H1 and H2), and 30 min after exercise (p < 0.05 for H1). Likewise, it enhanced coagulation immediately after exercise (CT1, p < 0.001; CT2, p < 0.01) and 30 min after exercise (CT1, p < 0.01; CT2, p < 0.05). On the other hand, Ex-90%VT had no significant effect on either platelet reactivity or coagulation.

Discussion

Shear stresses in normal arteries with laminar flow pattern are low and do not affect the circulating blood cells. However, in a severely stenosed artery with greatly reduced luminal diameter, or at a site of rupture of an ath-

Fig. 3. Effects of Ex-VT130% and Ex-VT90% on platelet reactivity (H1 and H2). Data were obtained before exercise (before), immediately after (after), and 30 min (rec. 30 min) after exercise at two different intensities corresponding to 90% (Ex-VT90%) and 130% (Ex-VT130%) of individual ventilatory threshold. Data were ana-

lyzed separately for each exercise test by one-way repeated-measures ANOVA (Ex-VT130: H1 and H2, $p < 0.05$). Values are expressed as the mean $\pm$ SEM. $\circ$ = Ex-VT90%; $\bullet$ = Ex-VT130%; * $p < 0.05$ as compared to before exercise (Fisher's PLSD).

Fig. 4. Effects of Ex-VT130% and Ex-VT90% on dynamic coagulation (CT1; CT2). Data were obtained before exercise (before), immediately after (after), and 30 min (rec. 30 min) after exercise at two different intensities corresponding to 90% (Ex-VT90%) and 130% (Ex-VT130%) of individual VT. Data were analyzed separately for

each exercise test by one-way repeated-measures ANOVA (Ex-VT130: CT1 and CT2, $p < 0.01$). Values are expressed as the mean $\pm$ SEM. $\circ$ = Ex-VT90%; $\bullet$ = Ex-VT130%; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared with before exercise (Fisher's PLSD).

erosclerotic plaque, shear stress can reach very high levels and usually is coupled with flow disturbances. At such high shear, platelets are activated, platelet-rich thrombus is formed, which may occlude the lumen [6, 18]. Thrombus formation is a critical determinant of the clinical outcome of acute cardiac and cerebral events such as angina, myocardial infarction or thrombotic stroke. Platelet function tests, which measure platelet response to high shear, are considered to be the best experimental approximation of human pathological conditions. Accumulating evidence shows that the measurement of shear-induced platelet reactivity is useful in assessing thrombotic tendencies [6, 7, 19]. In this study, we used a shear-induced platelet function test, hemostatology. Apart from induc-

ing platelet aggregation by shear forces, one of the unique features of hemostatology is the use of nonanticoagulated blood, which allows the simultaneous measurement of coagulation of flowing blood (dynamic coagulation). This is very important because arterial thrombosis is a multicellular event, involving not only platelets but also erythrocytes, leukocytes and the interaction of these cells [20, 21], which only occur at physiological calcium ion concentration [22, 23].

VT is defined as the work rate or oxygen intake just below the level at which metabolic acidosis and associated changes in gas exchange occur [24]. Along with increases in the intensity of exercise to higher than VT, an anaerobic component of metabolism increases significantly, and

this change causes the blood lactic acid level to increase. These changes induced by exercise at intensities higher than VT level strongly stimulate ventilatory function. Therefore, marked differences exist in the physiological response to exercise levels lower and higher than the VT level. Results of this study obtained in young healthy males reveal that thrombotic risk (enhanced platelet activity and hypercoagulation) occurs only after high-intensity exercise (intensity at 130%VT for 30 min) and this risk subsides 30 min after exercise. There was no thrombosis risk (platelet hyperreactivity or hypercoagulation) after low-intensity exercise (intensity at 90%VT for 30 min).

To the best of our knowledge, only few studies investigated the effect of acute exercise of various intensities on shear-induced platelet reactivity [9, 10]. Our present findings obtained immediately after exercise are in agreement with previous studies. These earlier studies suggested that hemoconcentration and increased plasma catecholamine concentrations could be involved in the exercise-induced enhanced thrombotic tendency [4, 12, 13, 25]. This view was supported by our previous findings showing elevated plasma norepinephrine concentrations coexisting with enhanced platelet reactivity [12, 13]. However, the present findings show that after high-intensity exercise, elevated plasma norepinephrine, erythrocyte counts, and hematocrit returned to preexercise levels but platelet and coagulation hyperactivity was sustained. Further, recent studies using hemostatometry revealed that sweating under high temperature and humidity increased platelet reactivity without increasing erythrocyte counts or hematocrit, and consuming drinking water after sweating prevented platelet hyperreactivity [Yamamoto et al., unpubl.]. It was reported that high-intensity exercise increased the density of α_2 -receptors on platelet membranes [26] and inhibited the response of platelets to nitric oxide (NO) [27]. These effects can offer a mechanism for the increased thrombotic tendency 30 min after exercise, observed in this study. However, the finding that high-intensity exercise increased NO release from platelets [28] contradicts to the above mechanism. Involvement of NO in exercise-induced increased thrombosis risk will be the subject of forthcoming investigations.

Another study of exercise of different intensities reported that the very significant (83% $\text{VO}_{2\text{max}}$) exercise-induced activation of coagulation was counterbalanced by the activation of the fibrinolytic system [29]. In contrast to this study, which used a factorial approach to assess the coagulation and fibrinolysis system, we measured the overall dynamic coagulation and spontaneous thromboly-

sis. We believe that only such global tests can reflect the physiological status of hemostasis, i.e. platelet function, coagulation and fibrinolysis.

Our findings show that low-intensity exercise does not cause any significant changes in the thrombotic status (fig. 3, 4). This is in accord with similar reports showing that exercise of light to moderate intensities does not increase the thrombotic risk caused by enhanced platelet adhesiveness, platelet release and thrombin production [9, 10, 29].

A recent epidemiological study suggested that the risk of a cardiac event after vigorous exercise increased in untrained individuals while in those accustomed to such vigorous exercise it increased the risk only slightly [30]. Subjects in the present study were young healthy males who regularly took moderate exercise and their mean $\text{VO}_{2\text{max}}$ was 49.7 ± 2.9 ml/kg/min. Such $\text{VO}_{2\text{max}}$ was greater than that measured in sedentary subjects but smaller than that of middle or long distance runners [31]. Our findings could have been different if the study had been performed in sedentary or more trained subjects. Nevertheless, the effect of the individuals' physical training on exercise-induced thrombotic risk needs further studies.

In conclusion, the present study demonstrated that only exercise at high intensity (intensity at 130%VT for 30 min), but not at VT or below, caused sustained platelet hyperreactivity and hypercoagulation, and as such, an increased risk of acute thrombotic event. Long-term low-intensity exercise has a beneficial effect on the clinical outcome of 'lifestyle-related diseases', including acute cardiac events [32–34]. Based on our present findings, we suggest that those who are at risk for coronary artery disease should exercise mainly at intensities below VT, and should refrain from high-intensity exercise.

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