Effects of Treadmill Exercise on Platelet Functions and Blood Coagulating Activities in Healthy Men

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SUMMARY

The effects of treadmill exercise (up to 85% of the predicted maximum heart rate) on platelet functions and coagulating activities were studied in 26 normal men. Blood sampling for the measurements were performed from the antecubital vein at rest, at 3, 6, 9, and 12 min during exercise, immediately postexercise, and at 6 and 30 min after exercise. Measurements for blood analysis included the following: platelet sensitivity and percent aggregation to ADP, platelet counts, plasma thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ levels, plasma epinephrine and norepinephrine levels, plasma fibrinogen level, activity of plasma antithrombin III, and of plasma factors VIII, IX, XI, and XII.

No significant changes were induced by dynamic leg exercise in platelet sensitivities and the maximum and 3-min percent aggregation. The platelet counts increased during exercise in platelet-rich plasma without a significant change in that in whole blood. During exercise, plasma thromboxane B_2 levels showed a tendency to increase, while plasma 6-keto-prostaglandin $F_{1\alpha}$ levels to decrease. Plasma epinephrine levels showed a tendency to increase and norepinephrine levels increased during exercise. Among coagulating factors, factor VIII activities and fibrinogen levels increased without altering activities of factors IX, XI, and XII. Antithrombin III activities also increased during exercise. In spite of significant changes in several coagulating factors, prothrombin time and partial thromboplastin time were not influenced by exercise.

In conclusion, dynamic leg exercise of a moderate to high intensity produced a significantly elevated plasma level of factor VIII, fibrinogen, antithrombin III, and catecholamines without affecting the hemostatic balance in normal subjects.

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Received for publication August 5, 1983.

Additional Indexing Words:

Treadmill exercise stress testings Platelet aggregation
Thromboxane B₂ 6-keto-prostaglandin F_{1α} Coagulating
activities

IT has been suggested that the disturbed dynamic balance among platelet function, coagulating activities and fibrinolysis may be a factor in the pathogenesis of atherosclerosis^{1),2)} and that platelets may play a part in atherogenesis and initiate arterial thrombosis by aggregation or by activating the coagulating factors. Effects of exercise on platelet functions, coagulating activities and fibrinolysis have been studied with isometric or dynamic exercise by many researchers.³⁾⁻¹³⁾ The alternation in platelet functions and coagulating activities during exercise was greater in patients with coronary artery disease than in normal subjects and was suggested to play a part in occurrence of angina pectoris and myocardial infarction by exercise.^{3),14)} Recently, the mechanisms of arachidonic acid metabolism have been clarified in platelets as well as in the endothelial cells of vessel walls, and prostaglandins derived from arachidonic acid such as thromboxane A₂¹⁵⁾ and prostacyclin^{16),17)} were found to be responsible for the control of platelet aggregation and vasoconstriction. Much attention has been paid to the role of prostaglandins in thrombotic conditions or during exercise.¹⁸⁾⁻²⁰⁾ However, the effects of exercise on platelet functions have not previously been studied in parallel with changes in blood coagulability, catecholamines, and prostaglandins.

In the present study, a systematic investigation was performed on normal subjects to clarify (1) what changes in platelet functions, prostanoids, catecholamines, and coagulating activities are induced by treadmill exercise, and (2) what the mechanism to maintain the hemostatic balance is.

MATERIALS AND METHODS

The materials consisted of 26 healthy male volunteers who had abstained from any medication for 2 weeks before the study. They ranged in age from 21 to 41 years with an average of 28.1 (± 4.1) years. According to the modified Bruce's protocol,²¹⁾ multistage treadmill exercise testing was performed to the target heart rate (85% of the predicted maximum heart rate). The testing was initiated at least 2 hours after a light lunch in the afternoon. Avoiding stagnation, blood sampling was obtained from the antecubital vein at rest immediately before exercise, at 3, 6, 9, and 12 min during exercise, immediately postexercise, and at 6 and 30 min after the exercise.

Nine ml of blood was aspirated into a polypropylene tube containing 1 ml

of 3.8% of trisodium citrate. Blood samples were immediately centrifuged at 800 rpm for 10 min at room temperature, and 2 ml of citrated platelet-rich plasma (PRP) was obtained as a supernatant. Residual samples were centrifuged at 3,000 rpm for 20 min, and then 3 ml of the supernatant was collected as platelet-poor plasma (PPP). According to the optical density method (O.D. method)^{22),23)} using PRP and PPP, platelet aggregation studies were performed. ADP of 4, 2, 1, and 0.5 μM was used as an aggregating agent. Aggregation curves were analysed to obtain the 3-min and maximum percent aggregation. Furthermore, the minimum ADP concentration (2^{-n} μM) to induce a biphasic aggregation curve was determined. The absolute value of the exponent (n) was defined as an index of platelet sensitivity to ADP. Counting platelets in PRP and citrated whole blood were made microscopically.

Five ml of blood was aspirated into a polypropylene tube containing 4.5 mM EDTA and 20 μM aspirin. Samples were centrifuged at 3,000 rpm for 20 min at 4°C to produce platelet-poor plasma for prostanoid assay. Radioimmunoassay for thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ was performed after extraction from the plasma with ethyl acetate and resuspension in 100 mM phosphate buffer (pH 7.4) containing 0.1% gelatin.²⁴⁾

Ten ml of blood for the determination of catecholamine levels were drawn into a polypropylene tube containing 4.5 mM EDTA. Samples were centrifuged at 3,000 rpm for 20 min at 4°C to produce platelet-poor plasma. Plasma epinephrine and norepinephrine levels were estimated according to the trihydroxyindol method described by von Euler et al²⁵⁾ and Mori²⁶⁾ using high-speed liquid chromatography.

Using residual citrated PPP, measurement of prothrombin time, partial thromboplastin time, and activities of factors VIII, IX, XI, and XII was made by the one-stage method which electronically measures the rate of changes in optical density. Biological activities of factors VIII, IX, XI, and XII were determined according to the methods of Hardisty,²⁷⁾ Rapaport,²⁸⁾ Horowitz,²⁹⁾ and Loeliger,³⁰⁾ respectively. Fibrinogen levels were determined by the single radial immunodiffusion method, and the biological activity of antithrombin III was determined by the quantichrom assay.

Paired t test was used for statistical comparisons. A p value of less than 0.05% was considered significant.

RESULTS

(A) Cardiovascular response to treadmill exercise

The mean duration for treadmill exercise was 13.2 (± 0.3) min, standard

error. The mean of heart rate, systolic blood pressure, and pressure-rate product at the peak of exercise was 159 (± 2)/min, 165 (± 4) mmHg, and 262 (± 8), respectively.

(B) Platelet functions and coagulabilities

The mean $\pm$ standard error of the measurements obtained was summarized in Tables I, II, and III.

Table I. Changes in Platelet Aggregation, Platelet Count, and Platelet-Related Prostaglandins

		during exercise					immediately after exercise	after exercise	
		at rest	at 3 min	at 6 min	at 9 min	at 12 min		at 6 min	at 30 min
Percentage of maximum platelet aggregation (%)	4 μ M of ADP	73.2 ± 3.3		70.3 ± 3.6		71.5 ± 4.9	68.0 ± 3.7	68.9 ± 2.8	71.3 ± 4.2
	2 μ M of ADP	58.3 ± 6.4		51.0 ± 5.5		63.0 ± 7.8	58.6 ± 6.4	56.5 ± 5.7	56.5 ± 5.8
	1 μ M of ADP	31.5 ± 4.7		34.2 ± 4.8		37.5 ± 4.4	35.8 ± 5.2	33.3 ± 4.1	31.1 ± 3.1
	0.5 μ M of ADP	16.7 ± 2.0		20.5 ± 3.2		22.5 ± 2.5	17.0 ± 2.2	16.9 ± 2.3	18.2 ± 2.8
	Platelet sensitivity	-1.09 ± 0.20		-1.09 ± 0.23		-0.88 ± 0.32	-1.05 ± 0.25	-1.09 ± 0.21	-1.18 ± 0.18
Platelet counts in citrated PRP ($\times 10^4$ /ml)		28.5 ± 1.7	34.3 $\pm 2.9^*$	31.4 $\pm 2.7^*$	32.5 ± 3.3	30.8 $\pm 3.2^{**}$	34.3 $\pm 2.1^{**}$	28.6 ± 2.1	25.7 ± 1.8
Platelet counts in citrated whole blood ($\times 10^4$ /ml)		18.6 ± 1.4	17.3 ± 0.9	18.3 ± 1.5	18.5 ± 2.0	18.4 ± 3.9	19.7 ± 1.4	19.1 ± 1.3	17.1 ± 1.1
Plasma thromboxane B ₂ (pg/ml)		80 ± 17		118 ± 27		80 ± 31	118 ± 21	160 ± 50	118 ± 27
Plasma 6-keto-PG F _{1α} (ng/ml)		1.12 ± 0.28		1.08 ± 0.21		1.13 ± 0.28	1.00 ± 0.17	0.95 ± 0.19	1.34 ± 0.36

Values are presented as mean $\pm$ standard errors.

* $p < 0.05$, ** $p < 0.025$, *** $p < 0.01$, **** $p < 0.005$ in comparison with values obtained before exercise.

Table II. Changes in Plasma Levels of Catecholamines

	during exercise					immedi- ately after exercise	after exercise	
	at rest	at 3 min	at 6 min	at 9 min	at 12 min		at 6 min	at 30 min
Plasma epinephrine (pg/ml)	75± 23	80± 25	94± 23	139± 27*	60± 38	130± 25	53± 17	51± 13
Plasma norepinephrine (pg/ml)	184± 28	356± 68***	379± 54****	583± 139***	482± 144	678± 135****	216± 46	147± 27

Values are presented as mean $\pm$ standard errors.

* $p < 0.05$, ** $p < 0.025$, *** $p < 0.01$, **** $p < 0.005$ in comparison with values obtained before exercise.

Table III. Changes in Blood Coagulability

	during exercise					immedi- ately after exercise	after exercise	
	at rest	at 3 min	at 6 min	at 9 min	at 12 min		at 6 min	at 30 min
Activity of factor VIII (%)	81±6	86±5	96±5*	86±9		103±6***	91±6	88±7
Activity of factor IX (%)	111±7	119±9*	107±6	116±10		117±9	119±8	116±9
Activity of factor XI (%)	136±9	170±15	156±17	159±16		135±11	135±14	133±10
Activity of factor XII (%)	121±10	113±10	120±10	119±12		124±10	126±9	123±10
Plasma fibrinogen (mg/dl)	248±9	263±11*	258±11	263±12	253±17**	268±10****	254±10	244±9
Activity of antithrombin III (%)	104±4	111±6	110±4****	112±6*	115±6*	115±4****	107±4	100±4
Prothrombin time (%)	104±2	104±2	104±2	103±2		103±1	104±1	105±2
Partial thromboplastin time (%)	98±6	91±7	94±7	93±8		94±4	92±5	92±4

Values are presented as mean±standard errors.

* $p<0.05$, ** $p<0.025$, *** $p<0.01$, **** $p<0.005$ in comparison with values obtained before exercise.

(1) Platelet aggregation

No significant changes were induced by exercise in the 3-min and maximum percent aggregation with 4, 2, 1, and 0.5 μM of ADP (Fig. 1) and also in platelet sensitivity. Platelet counts in PRP increased from 28.5 (± 1.7)/ mm^3 at rest to 34.3 (± 2.1)/ mm^3 immediately after exercise ($p<0.025$), but those in whole blood were not significantly influenced by exercise (Fig. 2).

Fig. 1. Changes in platelet sensitivity and max percent aggregation.

Fig. 2. Changes in platelet counts in PRP and citrated whole blood.

Fig. 3. Changes in plasma levels of thromboxane B₂ and 6-keto-prostaglandin F_{1α}.

(2) Prostanoids

Plasma thromboxane B₂ levels showed a tendency to increase postexercise, while plasma 6-keto-prostaglandin F_{1α} levels to decrease with exercise (Fig. 3).

(3) Catecholamines

The plasma level of epinephrine had a tendency to increase with exercise, showing a significant elevation at 9 min of exercise, while that of norepinephrine significantly increased as the intensity of exercise was augmented (Fig. 4). The latter rose from 184 (±28) pg/ml at rest to 678 (±135) pg/ml at the end point of treadmill exercise ($p < 0.005$). Both plasma catecholamines returned to the pre-exercise level at 6 min after exercise.

Fig. 4. Changes in plasma levels of epinephrine and norepinephrine.

Fig. 5. Changes in antithrombin III activity and fibrinogen level.

(4) Coagulating activities

The plasma level of fibrinogen was significantly elevated as the intensity of exercise increased (Fig. 5). It was $248 (\pm 9)$ mg/ml at rest and $268 (\pm 10)$ mg/ml immediately after exercise ($p < 0.005$). The antithrombin III activity also significantly rose from $104 (\pm 4)$ % at rest to $115 (\pm 4)$ % at the end point of treadmill exercise ($p < 0.005$). The factor VIII activity was significantly enhanced with exercise from $81 (\pm 6)$ % at rest to $103 (\pm 6)$ % at the end point of exercise ($p < 0.01$) (Fig. 6). The activity of factor IX showed

Fig. 6. Changes in activities of factors VIII and IX.

Fig. 7. Changes in activities of factors XI and XII.

a significant elevation only at 3 min of exercise, and that of factors XI and XII showed little changes with exercise (Fig. 7). In spite of the presence of significant exercise-induced changes in several coagulating factors, prothrombin time and partial thromboplastin time were not influenced by exercise (Fig. 8).

DISCUSSION

It has been reported by many researchers that dynamic or isometric exercise influenced the balance among platelet functions, coagulating activities and fibrinolysis in normal subjects and also in patients with a tendency to

Fig. 8. Changes in prothrombin time and partial thromboplastin time.

thrombo-embolism. However, there still existed controversies on the effects of exercise to these hemostatic parameters. The difference in the results of previous studies seems to be due to difference in the type of intensity of exercise, or the method used for the measurement of platelet functions, coagulating activities and fibrinolysis.

In the present study, we investigated platelet functions in treadmill exercise from the viewpoints of platelet aggregation and platelet-related prostaglandins in normal men. The platelet sensitivity as well as the percent aggregation assessed by the O.D. method were studied with ADP of 0.5, 1, 2, and 4 μ M. No significant changes were observed in these parameters and also in the plasma level of thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ during and after treadmill exercise with the target heart rate of 85% of the predicted maximum heart rate. It was confirmed that platelet function was not influenced by dynamic leg exercise of moderate to high intensity. These results are in accordance with some^{3), 6), 31)} but not all^{4), 5)} previous reports.

Yamazaki et al reported that in healthy volunteers there were no statistically significant changes in the percent ADP aggregation measured at 1, 3, 5, and 10 min after Master double two-step test.³⁾ Warlow et al reported a significant increase in the rate of the second phase of ADP- and epinephrine-induced aggregation both at 1 and 2 hours after strenuous running for 15 min in healthy men.⁴⁾ They also observed a highly significant increase in the mean platelet counts after exercise in both the citrated whole blood and PRP but no significant changes in platelet adhesion. On the other hand, Lee et al demonstrated a decreased platelet aggregation related to the level of exertion during and after multistage treadmill exercise in normal subjects and no rebound of platelet aggregation immediately after exercise.⁵⁾ In this report

the platelet aggregation study was performed using 40 μ M of ADP which was much higher concentration of ADP than ours, after normalization of platelet count. In these reports platelet aggregation was studied by O.D. method as in the present study. Thus, the effects of dynamic leg exercise on platelet function are still controversial.

Recently, a new microscopical method for the assessment of platelet sensitivity to ADP-aggregation was devised by Sano and co-workers.⁶⁾ This new method made it possible to assess platelet sensitivity without centrifugation, thus avoiding mechanical stress to platelets. It required only 0.8 ml of blood and inexpensive equipment. They showed that platelet sensitivity did not change with isometric exercise in healthy subjects. Later, they also studied the effects of isometric exercise on plasma von Willebrand's titer and beta-thromboglobulin levels in normal subjects, confirming no significant changes in these parameters.³¹⁾ However, the relationship between platelet aggregation and prostaglandins during exercise was not studied in the above reports.

In the present study, the platelet counts in PRP increased postexercise without a significant change in those in whole blood. There is a report that platelet counts increased in citrated whole blood and PRP following a short period of strenuous physical exercise.⁴⁾ The increase was too large to be attributable to the small increase in hematocrit and occurred so rapidly that it was unlikely to be due to increased platelet production. Bennett revealed significantly elevated platelet counts following strenuous exercise but no significant changes in them after mild exercise, suggesting that the increased counts of platelet may be due to their entry into the axial blood flow from the periphery of the vascular lumen.³²⁾ It was reported by Dawson et al that postexercise thrombocytosis could occur in splenectomized subjects.³³⁾ From these reports including ours, it is suggested that there is an increased mobilization of platelets following strenuous exercise, which will not, however, be induced by exercises of up to 85% of the predicted maximum heart rate. The observation in the present study that there was an increased count of platelets in PRP but not in whole blood, remains unanswered.

According to many reports, the plasma concentration of intrinsic catecholamines increases during exercise.^{12),34)} Epinephrine has three effects on platelet aggregation, namely induction of both the first and second aggregation, and potentiation of ADP aggregation,^{35),36)} while norepinephrine is less active than epinephrine on platelets. Elevated plasma levels of these substances is likely to enhance platelet aggregation. In the present study, plasma levels of epinephrine had a tendency to increase with exercise and those of norepinephrine significantly increased as the exercise intensity increased, without a significant change in ADP aggregation. From the results of our

study, it seems that the increased amounts of catecholamines in blood during exercise might be too small to activate platelet aggregation.

In the present study, dynamic leg exercise of moderate to high intensity showed a tendency to elevate the plasma level of thromboxane B_2 and to decrease that of 6-keto-prostaglandin $F_{1\alpha}$ in normal subjects, no significant changes being observed in platelet aggregation. Elevated plasma levels of thromboxane B_2 were found in both peripheral and coronary sinus blood in patients with angina pectoris and myocardial infarction,^{18),20)} and lowered levels of circulating prostacyclin were found in patients with ischemic heart disease.³⁷⁾ These reports including others¹⁹⁾ suggested that an elevated level of thromboxane A_2 and a lowered level of prostacyclin take part in thrombus formation.

The present study showed that treadmill exercise of moderate to high intensity induced a significant elevation in the plasma epinephrine and fibrinogen concentration as well as in the plasma activity of factor VIII and antithrombin III in normal subjects. There are a lot of reports concerning the effects of exercise on coagulating activities. Most of the data supported the concept that clotting was potentiated by exercise.^{7),8)} A number of exercise-related factors that would accelerate the clotting sequences included activation of the extrinsic pathways by release of tissue thromboplastin,⁹⁾ increased coagulation with lactate accumulation,¹⁰⁾ and increased concentration of plasma proteins owing to hemoconcentration and of specific clotting factors (factors VIII,¹¹⁾ V,¹⁰⁾ VII,¹³⁾ XII,¹³⁾ and fibrinogen¹²⁾). Factor VIII is believed to be identified as a co-factor for platelet adhesion,³⁸⁾ therefore this substance specifically takes part in thrombus formation. Hawkey and co-workers demonstrated that epinephrine, factor VIII, and fibrinogen increased after exercise with a bicycle ergometer in normal subjects, and suggested that an exercise-induced increase in factor VIII was mediated via adrenergic stimulation.¹²⁾ The storing site of factor VIII is thought to be on vascular endothelial cells.³⁹⁾ Elevation of factor VIII and fibrinogen levels during exercise in the present study seems to promote blood coagulability.

Antithrombin III, and inhibitor of the enzymatic activity of thrombin, is one of the important factors that regulate coagulability. A high incidence of thrombosis was pointed out in patients with familial antithrombin III deficiency.⁴⁰⁾ Therefore, a decreased antithrombin III activity tends to accelerate thrombo-embolism formation. However, the reports which studied the effects of exercise on this substance are very few. The present study showed an increase in antithrombin III, which seems to cancel the effects of the increased activity of factor VIII and the increased concentration of fibrinogen. As a result, prothrombin time and partial thromboplastin time

were not influenced by exercise.

In conclusion, a treadmill exercise of moderate to high intensity produced significant increases in factor VIII, fibrinogen and norepinephrine as well as antithrombin III, without affecting the hemostatic and coagulating balance in normal subjects. Effects of treadmill exercise of moderate to high intensity on hemostasis and coagulation seem to be little in normal subjects.

ACKNOWLEDGMENT

The authors express their gratitude to Prof. Itsuro Sobue, the First Department of Internal Medicine, School of Medicine, Nagoya University for his kind advice.

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