# Circulation

**Production of circulating platelet aggregates by exercise in coronary patients**

AG Kumpuris, RJ Luchi, CC Waddell and RR Miller

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/61/1/62.citation

# Production of Circulating Platelet Aggregates by Exercise in Coronary Patients

Andrew G. Kumpuris, M.D., Robert J. Luchi, M.D., Caroline C. Waddell, M.D.,
and Richard R. Miller, M.D.

with the technical assistance of James A. Brown and Yolanda A. Repinecz

SUMMARY    To determine the effect of exercise on the in vivo formation of circulating platelet aggregates in patients with severe coronary artery disease (CAD), platelet aggregate ratios (PAR) (normal 0.8–1.1) were measured immediately before and after treadmill exercise and 30 minutes after exercise in 17 CAD patients (group 1, mean age 55 years), 12 age-matched normal subjects (group 2), and 13 young normals (mean age 27 years, group 3). Coronary patients had lower resting PAR than group 3 ($0.79 \pm 0.05$ vs $0.98 \pm 0.03$; $p < 0.01$), while group 2 had an intermediate value $0.86 \pm 0.04$ ($p > 0.05$ vs CAD and group 3). Immediately after exercise, group 1 PAR declined from $0.79 \pm 0.05$ to $0.53 \pm 0.04$ ($p < 0.001$), while groups 2 and 3 were unchanged ($p > 0.05$; both $p < 0.001$ vs group 1); 30 minutes after exercise, PAR in group 1 rose to $0.66 \pm 0.05$ ($p < 0.05$ vs pre- and immediately postexercise); groups 2 and 3 remained unchanged vs pre- and immediately postexercise ($p > 0.05$; both $p < 0.001$ vs group 1). Six group 1 patients received 1300 mg aspirin daily for 10 days and repeated the protocol. Resting PARs were unchanged ($p > 0.05$) from resting values without aspirin. The exercise-induced decline in PAR was attenuated by aspirin: without aspirin, $0.73 \pm 0.02$ preexercise to $0.54 \pm 0.04$ postexercise; with aspirin, $0.73 \pm 0.03$ to $0.82 \pm 0.03$ ($p < 0.05$ vs no aspirin). These data indicate that platelet aggregation occurs with exercise in CAD. In addition, these data suggest that aspirin exerts a significant inhibitory effect on exercise-induced platelet aggregation in CAD patients.

THROMBOEMBOLISM has been implicated in the pathogenesis of both myocardial infarction and sudden death by some workers;[1, 2] this has been disputed by others.[3] Moreover, certain investigators have attributed a prominent role to platelets in the pathogenesis of atherosclerosis.[1, 4, 5] Thus, it has become important to better define abnormal platelet aggregation in patients with coronary disease. Current evidence suggests that intra-arterial platelet aggregation may occur spontaneously in vivo[2, 6] and that platelet survival is reduced in patients with coronary artery disease.[7] Pharmacologic agents that inhibit platelet aggregation normalize platelet survival.[8, 9]

Despite considerable interest in the role of platelets in the initiation of coronary thrombi, either transient or chronic, previous studies usually used in vitro techniques. Few investigators have studied the effect of exercise on platelet aggregation. In vivo platelet aggregates have been shown to be quite transient,[10] making it important to assay postexercise platelet aggregation with both an immediate and late postexercise measurement to avoid underestimating enhanced platelet aggregation. Finally, the effect of aspirin on platelet aggregation in coronary patients should be clarified.[11]

The present investigation was performed to define quantitatively whether platelet aggregates can be detected in blood drawn from the antecubital vein in patients with documented severe coronary artery disease at rest and in response to treadmill exercise. This investigation compared platelet aggregation ratios in coronary patients with those obtained in both age-matched controls as well as young normal controls. In addition, the effect of a therapeutic course of aspirin on platelet aggregation before and immediately after exercise was evaluated in the coronary patients.

## Methods and Materials

### Study Population

The study group included 42 male patients and subjects. Seventeen patients, mean age 55.5 years (range 33–65 years), with symptomatic coronary artery disease constituted group 1. Twelve of the 17 group 1 patients underwent coronary arteriography; eight patients had stenoses ($\geq 80\%$) of each of the three major coronary arteries, three patients had two-vessel disease and one patient had total occlusion of the left anterior descending coronary artery only. The remaining five patients had electrocardiographic evidence of transmural myocardial infarction. Twelve normal age-matched subjects, mean age 53 years (range 41–58 years), served as controls (group 2). Each subject in this group had a normal resting ECG, no history of heart disease and no major risk factor for coronary

artery disease. Five of the age-matched control subjects had previously undergone coronary arteriography and had normal coronary arteries. An additional 13 subjects 24–29 years old (mean 27 years) also served as normal controls (group 3). All were in good health, with no history of heart disease or other serious medical illness. No participant in the investigation received a platelet-inhibiting drug for at least 2 weeks before the study.

## Quantification of Platelet Aggregates

We used the method of Wu and Hoak to detect circulating platelet aggregates.[12] Venous blood was drawn from an antecubital vein in an identical manner from all study participants using a tourniquet in each. The blood (0.5 ml) was drawn into two separate siliconized syringes; syringe A contained 2 ml of buffered EDTA-formalin solution and syringe B contained 2 ml of buffered EDTA solution only. Solution A was prepared by mixing 1.5 parts 0.07 M $Na_2$ EDTA, 2.5 parts 4% formalin, and 6.0 parts tris-buffered saline at a pH of approximately 7.4. Solution B was prepared by adding 1.5 parts $Na_2$ EDTA (0.07 M) to 8.5 parts tris-buffered saline at a pH of 7.4.

The blood-solution mixtures were immediately transferred to silicon-coated plastic tubes and allowed to stand for 15 minutes at 22°C. The red cells were lysed using a Unopette containing ammonium oxalate and a phosphate buffer. The platelets were counted visually with a bright-line hemacytometer and a phase-contrast microscope. The results for each determination were expressed as a ratio:

Platelet aggregate ratio =

$$\frac{\text{Platelet count EDTA-formalin}}{\text{Platelet count EDTA}}$$

The normal range in this investigation was 0.8–1.1.[13]

## Study Protocol

Blood was drawn for the initial platelet assay in the fasting state while at rest. Each patient then underwent graded, multistaged treadmill exercise using the Bruce protocol.[14] Exercise was terminated in the group 1 patients at the onset of chest pain, exhaustion or upon obtaining 85% of maximal predicted heart rate. To insure a controlled standard hemodynamic stress, all subjects in groups 2 and 3 were required to achieve at least 85% of their maximal predicted heart rate to qualify for inclusion into the study. Blood was drawn for a second platelet aggregate ratio within the first minute after exercise and a third specimen was obtained 30 minutes after terminating the treadmill exercise test.

After completing the protocol, six patients in group 1 received aspirin (1300 mg/day) for 10 consecutive days. The protocol was then repeated.

Data were analyzed using the *t* test for paired and nonpaired values.

## Results

During treadmill exercise, the coronary artery disease patients constituting group 1 achieved a mean maximal heart rate of 68% of their predicted maximum. In contrast, all subjects in groups 2 and 3 achieved at least 85% of their maximal predicted heart rate (mean 88%; group 1 vs group 2, $p < 0.05$; group 1 vs group 3, $p < 0.05$). In group 1, 12 of 17 (71%) patients demonstrated an ischemic electrocardiographic response to exercise defined as horizontal or downsloping ST-segment depression of $\geq 0.1$ mV for at least 0.08 second beyond the J point. None of the control subjects in groups 2 or 3 developed ischemic electrocardiographic alteration, ($p < 0.05$ vs group 1).

At rest there was no difference ($p > 0.05$) in the mean platelet counts between the three groups. As previously reported, exercise increased the mean platelet count in each group.[15] However, a comparison of the percent rise and mean count of the three groups revealed no difference ($p > 0.05$).

The mean platelet aggregate ratio in group 1 at rest before exercise was 0.79 ± 0.05 (mean ± SEM) (range 1.05–0.29) (table 1). Nine of the 17 (53%) group 1 coronary patients had resting platelet aggregate ratios below the lower limits of normal. The average resting preexercise platelet aggregate ratio in the age-matched controls, group 2, was 0.86 ± 0.04 (1.02–0.64 range) ($p > 0.05$ vs group 1). Sixty-six percent of group 2 subjects had resting values within the normal range. The normal controls constituting group 3 had a resting mean platelet aggregate ratio value of 0.98 ± 0.03 ($p < 0.01$ vs group 1); all but one normal subject had resting values within the normal range.

Immediately after exercise, the mean platelet aggregate ratio in the group 1 coronary patients declined to 0.53 ± 0.04 ($p < 0.001$ vs group 1 preexercise) (table 1). The mean platelet aggregate ratios after exercise were similar ($p > 0.05$) in group 1 patients with and without exercise-induced electrocardiographic evidence of ischemia. In contrast, no significant ($p > 0.05$) reduction occurred with exercise in the mean platelet aggregate ratio in either groups 2 or 3 (table 1).

Thirty minutes postexercise the mean platelet aggregate ratio in group 1 increased to 0.66 ± 0.05 ($p < 0.05$ vs group 1 immediately postexercise) but remained depressed compared to the preexercise value of 0.79 ± 0.05 ($p < 0.05$). At 30 minutes after exercise, the platelet aggregate ratios in groups 2 and 3 remained at preexercise levels, 0.83 ± 0.05 and 1.0 ± 0.03, respectively (both $p < 0.001$ vs group 1; both $p > 0.05$ vs their resting preexercise values) (table 1).

The findings in the six group 1 coronary patients who received aspirin 1300 mg/day for 10 consecutive days and then repeated the exercise protocol are summarized in table 2. The mean platelet aggregate ratio in the six aspirin-treated patients at rest and 30 minutes after exercise did not differ significantly ($p > 0.05$) from the baseline pre-aspirin determinations. However, the decrease in the mean platelet aggregate

TABLE 1. *Exercise-induced Circulating Platelet Aggregates in Coronary Heart Disease Patients vs Normal Subjects*

| | Group 1 (n = 17) | Group 2 (n = 12) | Group 3 (n = 13) | p values | |
|---|---|---|---|---|---|
| Rest | 0.79 ± 0.05 | 0.86 ± 0.04 | 0.98 ± 0.03 | 1 vs 2 | >0.05 |
| | | | | 1 vs 3 | <0.01 |
| | | | | 2 vs 3 | >0.05 |
| Immediately after exercise | 0.53 ± 0.04 | 0.81 ± 0.06 | 0.91 ± 0.04 | 1 vs 2 | <0.001 |
| | | | | 1 vs 3 | <0.001 |
| | | | | 2 vs 3 | >0.05 |
| 30 minutes after exercise | 0.66 ± 0.05 | 0.83 ± 0.05 | 1.0 ± 0.03 | 1 vs 2 | <0.05 |
| | | | | 1 vs 3 | <0.001 |
| | | | | 2 vs 3 | <0.01 |

Values are mean ± SEM.
For definitions of groups 1, 2 and 3, see text.

ratio observed immediately following exercise was attenuated by aspirin therapy (table 2).

## Discussion

Our findings suggest that exercise will induce in vivo formation of circulating platelet aggregates in patients with severe coronary artery disease. Consistent with the findings of Wu and Hoak,[12] most (84%) of the control subjects in groups 2 and 3 had normal ratios at rest. Immediately postexercise, the young normals had normal values, as did 67% of the age-matched control subjects. However, four patients (33%) from this older control group had abnormal postexercise ratios that were consistent with platelet aggregate formation. Two of these four patients underwent coronary arteriography and were documented to have no coronary artery stenoses.

The aggregate ratios obtained from the patients with coronary artery disease consistently demonstrated the greatest degree of abnormality. Thus, at rest, nine of 17 (53%) of these patients had ratios below the lower limit of normal. While we can only speculate as to why such a large fraction of these patients had abnormal resting values, these data suggest that patients with severe coronary artery disease, some of whom have widespread vascular athero-

TABLE 2. *Effect of Aspirin on Exercise-induced Platelet Aggregation in Coronary Artery Disease*

| | Group 1 No aspirin | Group 1 Aspirin |
|---|---|---|
| Rest | 0.75 ± 0.02 | 0.73 ± 0.03 |
| Immediately after exercise | 0.54 ± 0.04 | 0.82 ± 0.03* |
| 30 minutes after exercise | 0.71 ± 0.03 | 0.80 ± 0.03 |

*$p < 0.05$ vs no aspirin immediately after treadmill exercise.
Group 1 is defined in text.

sclerosis, may have abnormal circulating platelet aggregates related to the diffuse nature of the disease. Immediately after exercise, 16 of 17 (94%) were abnormally low. Most important, although the mean ratio tended to decrease in each of the three groups immediately after exercise, the decline was statistically significant ($p < 0.001$) only in the patients with coronary artery disease, indicating that in this select population, exercise induces platelet aggregate formation. In addition, the platelet aggregate ratios were lower ($p < 0.05$) in the coronary patients at each measurement period than in the young controls and significantly lower ($p < 0.05$) than in the age-matched controls at the immediate and 30-minute postexercise measurement intervals.

Prior attempts to define abnormal in vitro platelet aggregation after standardized exercise testing have yielded conflicting results. Yamazaki et al.[16] and Levites and Haft[17] found increased aggregation immediately after exercise testing in patients with coronary disease. However, other studies did not confirm these observations.[18, 19] The reasons for the disparity in results is unclear; however, the interval from the completion of the exercise test to the time of phlebotomy may have differed between the various studies. Particularly noteworthy is the recent observation that aggregates will disaggregate within 6–10 minutes.[10] Thus, the exercise-induced hyperaggreable state may be short-lived. This observation is supported in the present study by our findings that 30 minutes after exercise, the coronary patients uniformly had ratios that were higher than the immediate postexercise values and approximated the resting values. These data support the concept that enhanced aggregation is a transient phenomenon and that in vivo platelet aggregates are capable of relatively rapid disaggregation.

Normally, platelets do not aggregate spontaneously in vivo. However, if a suitable stimulus is present, aggregation can occur. Postulated mechanisms include exposure to collagen in atherosclerotic lesions;[8, 20] platelet damage secondary to shear stress;[21] post-

obstructive luminal turbulence resulting in increased platelet-platelet interactions;[10] and enhanced or abnormal responsiveness to circulating catecholamines.[22] As blood flow increases as a result of the hemodynamic response to exercise, each of these mechanisms would be enhanced and may cause the production of circulating aggregates in the coronary artery disease patients. Mehta et al. recently demonstrated that atrial pacing-induced tachycardia will increase platelet aggregation.[23] Subclinical atherosclerotic lesions, which are beyond the limits of angiographic detection, or peripheral vascular disease, may also trigger one or several of the proposed mechanisms. This explanation may apply to several of the age-matched normals, some with normal coronary arteries, who developed platelet aggregates immediately after exercise.

Finally, we do not know if in vivo tests do indeed detect circulating platelet aggregates or if aggregates form after phlebotomy. It has been suggested that the method of Wu and Hoak may actually be detecting platelet hypersensitivity with consequent enhanced in vitro formation of platelet aggregates.[11, 24] Despite these unresolved questions, the technique does appear to clearly identify a population of abnormal platelets that occur in patients with vascular disease.

Of special importance in the present investigation is the effect of aspirin on the exercise-induced platelet aggregate ratio in patients with coronary artery disease. The decline in the mean aggregate ratio was attenuated by a 10-day course of aspirin ($p < 0.05$). These findings suggest that in certain patients with coronary artery disease, there is increased activity of the arachidonate pathway after exercise, which results in increased platelet aggregation.[25]

## Acknowledgment

## References

1. Mustard JF, Packham MA: The role of blood and platelets in atherosclerosis and the complications of atherosclerosis. Thromb Diath Haemorrh 33: 444, 1975
2. Haerem JW: Mural platelet microthrombi and major acute lesions of main epicardial arteries in sudden death. Atherosclerosis 19: 529, 1974
3. Roberts WC, Buja LM: The frequency and significance of coronary arterial thrombi and other observations in fatal acute myocardial infarction: a study of 107 necropsy patients. Am J Med 52: 425, 1972
4. Ross R, Glomset JA: The pathogenesis of atherosclerosis. N Engl J Med 295: 396, 1975
5. Ross R, Glomset JA, Harker L: Response to injury and atherosclerosis. Am J Pathol 86: 675, 1977
6. Haft JI, Fani K: Intravascular platelet aggregation in the heart induced by stress. Circulation 47: 353, 1973
7. Steele PP, Weily HS, Davis H, Genton E: Platelet function studies in coronary artery disease. Circulation 48: 1194, 1973
8. Salky N, Dugdale M: Platelet abnormalities in ischemic heart disease. Am J Cardiol 32: 612, 1973
9. Steele PP, Rainwater J, Vogel R, Genton E: Platelet-suppressant therapy in patients with coronary artery disease. JAMA 240: 228, 1978
10. Folts JD, Crowell EB, Rowe GG: Platelet aggregation in partially obstructed vessels and its elimination with aspirin. Circulation 54: 365, 1976
11. Mustard JF, Packham MA, Avery GS (eds): Cardiovascular Drugs, vol 3. Anti-Thrombotic Drugs. Baltimore, University Park Press, 1978
12. Wu KK, Hoak JC: A new method for the quantitative detection of platelet aggregates in patients with arterial insufficiency. Lancet 2: 924, 1974
13. Hoak JC: Discussion. Circulation 53 (suppl I): I-70, 1976
14. Bruce RA, Hornsten RT: Exercise stress testing in evaluation of patients with ischemic heart disease. Prog Cardiovasc Dis 11: 371, 1969
15. Dawson AA, Ogston D: Exercise-induced thrombocytosis. Acta Haematol 42: 241, 1969
16. Yamazaki H, Kobayashi I, Shimamato T: Enhancement of ADP-induced platelet aggregation by exercise test in coronary patients and its prevention by pyridinolcarbonate. Thromb Diath Haemorrh 24: 438, 1970
17. Levites R, Haft JI: Effects of exercise induced stress on platelet aggregation. Cardiology 60: 304, 1975
18. Frishman W, Christodoulou J, Weksler B, Smithen C, Horwitz S, Killip T: Platelet aggregation in angina pectoris: effect of exercise induced stress. (abstr) Am J Cardiol 33: 137, 1974
19. Davis JW, Lewis DH, Phillips PE: Effects of aspirin on exercise induced angina. Clin Pharmacol Ther 23: 504, 1978
20. Marcus AJ: Platelet function (third of three parts). N Engl J Med 280: 1330, 1969
21. Brown CH, Leverett LB, Lewis CW, Alfrey CP, Hellums JD: Morphological, biochemical, and functional changes in human platelets subjected to shear stress. J Lab Clin Med 86: 462, 1975
22. Carvalho AC, Colman RW, Lees RS: Platelet function in hyperlipoproteinemia. N Engl J Med 290: 434, 1974
23. Mehta J, Mehta P, Pepine CJ: Platelet aggregation in aortic and coronary venous blood in patients with and without coronary disease. 3. Role of tachycardia stress and propranolol. Circulation 58: 881, 1978
24. Packham MA: Methods for detection of hypersensitive platelets. (Stuttg) Thrombos Haemostas 40: 175, 1978
25. Roth GJ, Stanford N, Majerus PW: Acetylation of prostaglandin synthetase by aspirin. Proc Nat Acad Sci USA 72: 3073, 1975