Upright posture and maximal exercise increase platelet aggregability and prostacyclin production in healthy male subjects

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Abstract

Background—It is well accepted that heavy physical exertion can trigger the onset of myocardial infarction, but the mechanism is uncertain. As platelet and endothelial function play an important role in thrombotic events, platelet and prostacyclin responses to maximal treadmill exercise were studied.

Methods/Results—The study subjects were 40 healthy men, mean (SEM) age 29 (5) years. Platelet aggregation was measured on a four channel aggregometer. Plasma 6-keto-prostaglandin F_1α was analysed using an enzyme immunoassay technique. Upright posture and exercise produced an increase in platelet aggregability, as indicated by a fall in the threshold concentration of adrenaline (epinephrine) from 7.6 (1.5) µM at rest to 4.3 (1.0) µM after exercise (p = 0.002). The collagen lag time became significantly shorter with exercise (from 79.1 (3.1) seconds at rest to 71.9 (2.6) seconds after exercise, p = 0.003). Exercise was also associated with a 55% increase in plasma 6-keto-prostaglandin F_1α (from 38.1 (75%CI 29.0 to 46.5) pg/ml at rest to 59.2 (47.3 to 66.8) pg/ml after exercise, p<0.001).

Conclusions—In healthy male subjects, upright posture and maximal exercise increased platelet aggregability but this increase was counteracted by an increase in prostacyclin production. In patients with endothelial dysfunction, a reduced prostacyclin response to exercise may promote a transient prothrombotic imbalance that may trigger cardiovascular disease onset.

Keywords: exercise; platelet; collagen; 6-keto-prostaglandin F_1α; cardiovascular disease

Heavy physical exertion has been shown to trigger the onset of acute myocardial infarction and sudden cardiac death, particularly in sedentary people. Platelet activation plays an important role in the pathogenesis of these acute coronary ischaemic syndromes. Firstly, myocardial infarction and sudden cardiac death are often associated with platelet rich thrombi or platelet aggregates. Secondly, biochemical markers of platelet activation are increased during episodes of acute cardiac ischaemia. Finally, antiplatelet agents have been shown to reduce the incidence of cardiovascular ischaemic events.

Stress induced hypercoagulability may be a link between a stressor and an acute coronary event. The effect of exercise on platelet aggregability remains uncertain, with reports indicating an increase, no change or even a decrease in platelet reactivity. The effect of exercise on platelet adhesion is also poorly defined, in part because of the use of methodology that could not reliably distinguish platelet adhesion from aggregation in those studies.

We therefore studied 40 healthy men to determine the effect of maximal treadmill exercise on platelet aggregability and adhesion. In addition, we also determined the effect of exercise on thromb oxide and prostacyclin production.

Methods

STUDY POPULATION

The study protocol was approved by the institutional review board of the Deaconess Hospital. The investigation was in accordance with the Declaration of Helsinki. Informed consent was obtained from all subjects. Exclusion criteria and subject characteristics have been described in detail previously. The data reported here are from responses observed in the placebo phase of a study of the effect of aspirin dosage and enteral coating on platelet reactivity. Whereas the preceding paper focused on the effect of aspirin on platelet aggregability and prostacyclin, this one provides information on the effect of exercise on platelet reactivity.

Forty healthy untrained men completed the study. The mean (SEM) age was 29 (5) years. At screening, a medical history was taken and subjects underwent a physical examination, electrocardiogram, and blood drawing for platelet aggregation studies, blood count, and biochemistry profile. Those who met enrolment criteria returned within two weeks of screening for study. After a standardised breakfast, subjects rested for 30 minutes in the supine position and then performed a maximal exercise treadmill test using the Bruce protocol. Blood samples were obtained immediately from an antecubital vein before and after exercise while the subjects were in the supine position. Blood was withdrawn in a syringe containing 3.8% sodium citrate (9:1, v/v). Platelet rich plasma was separated immediately after blood withdrawal by centrifugation for 10 minutes at 160 g.

PLATELET AGGREGATION

Platelet aggregation was measured on a four channel aggregometer at 37°C by the method of Born.
concentrations (0.01–30 µM) and a fixed concentration of collagen (0.19 mg/ml). At each time point, the following parameters were obtained: (a) the lowest concentration of adrenaline and ADP required to produce a biphasic response with greater than 50% aggregation (threshold concentration); (b) the collagen lag time, defined as the time interval in seconds from the addition of 0.19 mg/ml collagen to the commencement of aggregation.

**PLATELET ADHESION**

Platelet adhesion was measured using a parallel plate flow chamber in which platelet rich plasma was perfused at a constant flow rate to obtain a shear stress of 26 s⁻¹. The modified Hele-Shaw chamber consisted of a glass slide coated with fibronectin, attached to the flow chamber by a perimeter vacuum pump. The apparatus was mounted on an Olympus inverted microscope equipped with Hoffman Contrast. The adhesion process was recorded by taking photographs of the coated surface every 30 seconds. The total number of platelets adherent to the slide was counted after 10 minutes of flow. Platelet analysis was completed within two hours of the blood collection.

**ASSAY OF PLASMA 6-KETO-PROSTAGLANDIN F₁₆ AND THROMBOXANE B₂**

Blood samples were withdrawn and mixed immediately with citrate/citric/dextrose, containing 20 µM indomethacin in a ratio of blood to anticoagulant of 6:1. The blood samples were then placed on ice and centrifuged at 2000 g at 4°C for 15 minutes. The platelet poor plasma was removed and frozen at −80°C for later analysis using an enzyme immunoassay technique. The interassay and intra-assay coefficients of variation were 9.5% and 7.2% for 6-keto-prostaglandin F₁₆ and 8.6% and 5.6% for thromboxane B₂.

**STATISTICAL ANALYSIS**

The paired t test, or Wilcoxon Signed Rank test when the variables were not normally distributed, were used to compare results before and after exercise. All data are reported as mean (SEM) except for 6-keto-prostaglandin F₁₆ and thromboxane B₂ levels, which are expressed as median (75% confidence interval) because the distribution was abnormal. A two tailed p value <0.05 was regarded as statistically significant.

**Results**

The mean (SEM) exercise duration in these healthy subjects was 13.5 (0.3) minutes. Heart rate increased significantly from 78 (2) beats per minute at baseline to a peak of 189 (1) (p<0.0001). Systolic arterial pressure also increased from 117 (2) mm Hg at baseline to 169 (2) mm Hg at peak exercise (p<0.0001). Diastolic blood pressure decreased with exercise from 77 (1) at baseline to 73 (1) after exercise (p = 0.0009).

**PLASMA THROMBOXANE B₂ (TABLE 1)**

Exercise tended to increase plasma thromboxane production, from 54.6 (36.6 to 81.7) pg/ml at rest to 83 (9.4) pg/ml after exercise (p = 0.15).
Values are mean (75% confidence interval).

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Exercise</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-Keto-prostaglandin F₁α</td>
<td>38.1 (29.0 to 46.5)</td>
<td>59.2 (47.3 to 66.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Thromboxane B₂</td>
<td>54.6 (36.6 to 81.7)</td>
<td>73.5 (42.8 to 93.4)</td>
<td>0.15</td>
</tr>
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**Exercise effect on platelet aggregability and prostacyclin production in men**

Correction for changes in platelet count modified the effect on platelet aggregability. Whereas studies that do not correct for the increased platelet count after exercise tend to show an increased aggregability, those that use a standardised platelet count mostly show no change. In our study, neither the absolute platelet count nor the change in count with exercise correlated with platelet aggregability, suggesting that the increased platelet number with exercise is not the sole mechanism of the observed increase in platelet aggregability.

**Discussion**

In this study of healthy men, upright posture and maximal treadmill exercise significantly increased the platelet reactivity to adrenaline and collagen induced aggregation, thereby creating a prothrombotic change. However, exercise in these healthy subjects also produced a counteracting antithrombotic increase in prostacyclin production.

Although regular physical activity is protective against cardiovascular disease, several epidemiological studies indicate that heavy exertion, particularly in sedentary people, may trigger acute myocardial infarction and sudden cardiac death. The increased incidence of myocardial infarction and sudden cardiac death in the morning also coincides with increased sympathoadrenal activity and increased platelet aggregability. Stress induced platelet activation may be a link between exertion and an acute coronary event.

**Effect of maximal exercise on platelet aggregability**

The effect of exercise on platelet aggregability is controversial, with varying results being obtained. This variation is due in part to differences in exercise protocol, subject characteristics, or method of assessment of platelet activation. In most, but not all, studies, short strenuous exercise has been found to increase platelet aggregability, whereas mild or moderate exercise tends to have no effect on platelet reactivity or may even decrease platelet aggregability, whereas in sedentary subjects, exercise may even decrease platelet aggregability. Stress induced platelet activation may be a link between exertion and an acute coronary event.

**Effect of maximal exercise on platelet adhesion**

In contrast with platelet aggregability, platelet adhesion as assessed by fibronectin adherence was not influenced by maximal exercise in our study. Several static and dynamic systems have been used to measure platelet adhesion. These include the use of glass beads, a rotating glass bulb, and a glass-wool filter technique. With these methods, platelet adhesiveness has been variably reported to be increased, unchanged, or even decreased by exercise. As the assays do not accurately distinguish platelet adhesion from aggregation, the conclusions drawn from the studies are uncertain. In our modified Hele-Shaw flow chamber system, we were able to accurately monitor and quantify platelet adhesion to a fibronectin coated surface.

In contrast with our results, Wang and colleagues, using a similar parallel plate chamber system, found an increase in platelet adhesion with strenuous exercise. However, there are several methodological differences between their system and ours. Firstly, in their static chamber system, platelet rich plasma was gently infused into the chamber and kept there for 12 minutes to allow platelets to settle on the fibrinogen coated surface. The chamber was then flushed with Tyrode’s/Hepe buffer for five minutes. In contrast, we continuously perfused platelet rich plasma at a shear rate of 26 s⁻¹ and dynamically monitored platelet adhesion as it might occur on a plaque exposed to...
blood flow. Secondly, as the index of platelet adhesion, they used the slope of attached platelets versus shear stress, whereas we used the absolute number of platelets that adhered to the slide. Thirdly, they used fibrinogen as a coating material whereas we used fibronectin.

**MAXIMAL EXERCISE AND PROSTACYCLIN PRODUCTION**

In our study, prostacyclin levels significantly increased with maximal exercise. Prostacyclin is a potent vasodilator and platelet inhibitor. This increase in prostacyclin, as has been shown by other investigators, would tend to balance any prothrombotic tendency induced by strenuous exercise. In addition, strenuous exercise has been shown to significantly increase fibrinolytic activity, which can protect against thrombosis. However, these protective responses may be attenuated in subjects with coronary heart disease who show reduced prostacyclin release or fibrinolytic response and greater thrombopoeic production after stress. In the presence of a ruptured plaque and collagen exposed to flowing blood, the exercise induced increase in platelet aggregability in the presence of reduced prostacyclin release may promote occlusive thrombosis and acute myocardial infarction.

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