PATHOPHYSIOLOGY AND NATURAL HISTORY

THROMBOSIS

Inhibition of platelet aggregability by moderate-intensity physical exercise: a randomized clinical trial in overweight men

RAINER RAURAMAA, M.D., PH.D., JUKKA T. SALONEN, M.D., PH.D., KARI SEPPÄNEN, M.SC., RIITTA SALONEN, M.D., JUHA M. VENÄLÄINEN, M.D., MERJA IJANAINEN, M.SC., AND VILJO RISSANEN, M.D., PH.D.

ABSTRACT It has been postulated that platelet function plays an important role in the initiation of atherosclerosis. Currently there are no definitive data on the longer-term effects of regular physical exercise on platelet function in humans. We assessed the influence of regular moderate-intensity physical exercise (brisk walking to slow jogging) on platelet aggregation in a population-based sample of middle-aged, overweight, mildly hypertensive men in eastern Finland. In this controlled study, we evaluated the net effect of exercise on platelet aggregation by studying changes in optical density and ATP release in platelet-rich plasma. A significant inhibition of secondary platelet aggregation from 27% to 36% was observed in the men taking regular exercise. These findings give new insight into the possible protective effects of exercise against the risk of ischemic heart disease.


INTERACTIONS among platelets, lipoproteins, and arterial vessel wall have been postulated to be centrally involved in the development of atherosclerosis. Increased low-density lipoprotein (LDL)-cholesterol, causing endothelial injury, together with an increased rate of LDL infiltration promote platelet aggregation. The ensuing smooth muscle cell proliferation and cholesterol accumulation together with the secretion of connective tissue matrix finally initiate the formation of atherosclerotic plaque. Furthermore, there is evidence of thrombosis in coronary arteries both in myocardial infarction and sudden cardiac ischemic death, possibly caused by continuous arterial microemboli.

The initial phase of thrombosis is thrombocyte aggregation, preceded by the activation of platelets. An increased activation of platelets has been observed in patients with ischemic heart disease (IHD) during physical exercise, although not consistently. Temporarily increased platelet aggregation has been observed in healthy young men after both short-term and long-term strenuous running exercise. At present, no experimental data are available on the longer-term effects of physical training on platelet aggregability in humans. The overall purpose of this study was to investigate the effects of regular low-intensity physical exercise on risk factors for IHD and on platelet function in vitro. Our results demonstrate that exercise training can lower the sensitivity of platelets to the action of agonist, a potentially beneficial effect.

Methods

Subjects. The subjects for this study were men aged 30 to 49 years recruited from the random population sample examined in the 10 year follow-up survey of the North Karelia Project in 1982. Of approximately 2000 subjects examined, 195 were selected on the basis of the following criteria: residence in the city of Kuopio or its 13 surrounding communities, a sitting diastolic blood pressure between 95 and 109 mm Hg, no history of antihypertensive medication, and a body mass index of at least 24 kg/m². In the beginning of the lead-in period, the purpose of the study was explained to participants and verbal informed consent was obtained. During this 6 month lead-in period, blood pressure was measured four times at an interval of 4 to 6 weeks. In addition, physical work capacity and biochemical risk factors for IHD were determined once before the final enrollment of the subjects for the baseline examinations preceding the intervention.

During this pre-study period, 71 men were excluded because of a decrease in diastolic blood pressure (supine < 90 mm Hg or sitting < 95 mm Hg) or in body mass index to below 24 kg/m² or
because of initiation of drug treatment for hypertension. Furthermore, 44 men refused to continue to participate during the lead-in period. The final study group consisted of 80 men who fulfilled the inclusion criteria, and they were randomly assigned to one of three groups.

Twenty-six men were assigned to an exercise group, 27 to a reference group, and 27 to a dietary intervention group. The men in the exercise and reference groups were given placebo capsules containing a minute amount of olive oil, whereas those in the dietary group received capsules containing eicosapentaenoic and docosahexaenoic acid. The data on the dietary group will be presented elsewhere. Three subjects dropped out of the exercise group because of lack of motivation. Furthermore, one subject suffered from sciatica in the beginning of the training program and was unable to continue. In another subject the resting blood pressure increased over the exclusion limit during the intervention. He was put on drug therapy and his data are not included here. In addition, there were two subjects in whom venipuncture was unsatisfactory at the end of the intervention and data are missing. Thus 19 members of the exercise group completed the platelet aggregability studies. In the reference group there were two subjects who did not appear in the laboratory for the blood sampling at the end of the intervention. The reasons for their failure to appear are unknown. Thus the reference group included 25 subjects. The subjects made two visits to the laboratory both at baseline (after the 6 month lead-in period) and after the training period. Physical examination, basic anthropometric measurements, and determination of maximal oxygen consumption (VO2max) were performed at the first clinic visit. In addition, the subjects were given instructions for keeping dietary records beginning the day after the first visit to the laboratory and over the following 4 days. Dietary logs were checked by the nutritionist during the second laboratory visit 1 week later, at the time of venipuncture. The data on food consumption were analyzed by computer.11

Exercise testing and intervention. A maximal exercise test was performed on an electrically braked bicycle ergometer (Siemens Elema 380B, Siemens Elema AB); oxygen consumption was determined with the aid of respiratory gas analyses, averaged every 30 sec (Oxycon-4, Mijnhardt). The workload was increased by 10 W every 30 sec until the maximum level (subject-exhaustion or a respiratory exchange ratio [VO2/VO2] over 1.0) was reached. The electrocardiogram was recorded continuously during the test and for at least 6 min during recovery.

The subjects assigned to the exercise group were given an individually prescribed progressive training program for 12 weeks based on their VO2max. Toward the end of the intervention, the training intensity achieved 45% to 55% of the VO2max; exercise consisted of brisk walking and slow jogging five times a week, 45 to 60 min per session. Each exercise session included warming-up and cooling-down phases. The prescribed training intensity was controlled by a portable heart rate recorder (Sport Tester, Escotec).

Biochemical analyses. To avoid the possible immediate responses to physical exercise, the subjects were asked to abstain from any additional physical activity after the maximal exercise test, which was done 1 week before blood sampling, both before and after the intervention period. In addition, they were asked not to take any anti-inflammatory analgesics during the 2 preceding weeks and to abstain from alcohol 1 week before blood sampling. Their compliance, based on questioning before venipuncture and on the negative results of screening for salicylates13 in the serum at the end of the intervention, was excellent. Venipuncture took place after the subjects were in the supine position for half an hour, following a 12 hr fast, and blood was collected without a tourniquet into siliconized vacuum tubes (Terumo Venoject) that contained 0.31% trisodium citrate as the anticoagulant. Thrombocyte aggregation in samples of platelet-rich plasma (PRP) were measured as a function of time by the Whole Blood Aggro-Meter Model 500 (Chrono-log Co.). During measurements, both PRP and platelet-poor-plasma (PPP) were kept at 37°C. Luciferase-luciferin reagent (Chrono-log Co.) was added into the sample of PRP (1.0 ml) and the PPP sample was adjusted with 100 μl of 0.9% NaCl to the same basic optical density. As an aggregating agent, 1.0 mM ADP was used, and after 10 min the luminescence channel was calibrated with 2.004 mM ATP (Sigma Chemical Co.). Final concentrations of ADP were 2.27 to 9.01 μm/liter. Each subject’s samples before and after intervention were always handled with the same dose of aggregating stimulus. The variables of platelet aggregation (figures 1 and 2) were determined visually by the same technician, who had no knowledge of the randomization. Serum thrombomax was measured by radioimmunoasay as explained previously,13 and the results were calculated per platelet concentration of 100 × 109/liter.

Statistical methods. The effect of the exercise intervention was estimated as the net difference between the mean values before and after the period of regular training minus the corresponding difference for the reference group. The statistical significance was tested by Student’s t test comparing the means of the changes in the two groups. The long-term effects of exercise training were also estimated and tested by the analysis of covariance with adjustments for changes in dietary intake of polyunsaturated/saturated fatty acids (P/S ratio), and in alcohol consumption and for the baseline value of the respective aggregation variable. The association between the changes in serum thromboxane and platelet aggregation during physical exercise was analysed by regression analysis. All statistical inferences were based on two-tailed tests.

Results

There were no differences between the exercise and reference groups, respectively, in the mean baseline values of body mass index (27.7 vs 27.8 kg/m2), VO2max (38.4 vs 36.1 ml/kg/min), alcohol intake (2.6 vs 3.1 g/day), or P/S ratio (0.26 vs 0.27). The net differences of these variables changed insignificantly, the greatest change being an increase of 9% in the VO2max in the exercise group.

At baseline there were no differences in the aggregation measures (table 1) between the groups. Primary (reversible) aggregation of platelets did not change in either of the two groups during the training period. The secondary (total minus primary phase) aggregation time was reduced by 52% in the exercise group and by 17% in the reference group, giving a net effect of exercise of 36% (p = .019). The extent (mV) of secondary platelet aggregation was reduced, i.e., optical density increased by 70% in the exercise group, and by 41% in the reference group, for a net effect of 27% (p = .211). The amount of ATP released was reduced by 60% in the exercise group and by 19% in the control group, giving a 32% (p = .080) net effect of training.

Adjustment for covariates (see Methods) increased the statistical significance of the net differences for the
FIGURE 1. Platelet aggregation in an exercising subject before (A) and after (B) intervention. Addition of ADP starts the reaction. The onset of the irreversible (secondary) aggregation was determined on the basis of the beginning of ATP secretion in the release reaction. This subject's secondary aggregation time in optical channel before the intervention is representative of the respective group mean value.

secondary aggregation time \( p = .017 \) as well as for the increase in optical density \( p = .056 \).

Regression of the change in secondary platelet aggregation (change in mV) on change in serum thromboxane yielded a slope of 0.115 \( p = .0001 \) in the exercise group compared with a slope of 0.037 \( p = .028 \). Thus in the exercise group the slope was three times \( p = .004 \) greater than that in the reference group.

Discussion

Our data show that increased regular physical activity of low-to-moderate intensity results in reduced platelet aggregability in vitro in middle-aged, overweight, untreated, mildly hypertensive men compared with men whose physical activity level remains stable. The main finding is that regular physical exercise seems to have a long-term inhibitory effect on platelet aggregation, which persists for several days. This is in contrast to the immediate proaggregatory effect of acute physical exercise, reported to last for a few hours after exercise.\(^9\) Although platelet aggregation is only one part in the complex "cascade of thrombogenesis," these data may have important public health implications. They may fill some of the gaps in understanding how regular physical activity protects against ischemic vascular diseases.\(^{14-17}\)

Our study population was derived on the basis of
objective geographic and medical criteria from a random sample in eastern Finland. To avoid temporary changes toward more healthy behaviors after having been recruited for a clinical trial, the subjects underwent several examinations during the 6 month lead-in phase. While this long period stabilized the experimental conditions, it also allowed screening of potential dropouts. There remained 124 eligible men, of whom 65% participated in the study. We believe that this objective procedure and high response rate minimized selection bias.

We used before and after the training program the same individually pretested concentration of ADP as the aggregating stimulus, which gave the biphasic reaction. This procedure is justified by the observation that the primary platelet aggregation remained constant before and after the experimental period in both groups, whereas the secondary aggregation changed after the exercise program. Accordingly, the release reaction, by which also the primary and secondary phases could be separated, was not masked by addition of the stimulant. It is important to note that this study dealt with long-term effects of regular exercise training rather than short-term effects of physical exercise. Thus, because of the study design, in vitro instead of in vivo measurement of platelet function was considered appropriate.

Platelet aggregation showed inhibition after the ex-
TABLE 1
Secondary platelet aggregation in the exercise (n = 19) and reference (n = 25) groups initially and after 12 weeks of physical training

<table>
<thead>
<tr>
<th></th>
<th>Exercise group</th>
<th>Reference group</th>
<th>Statistical significance&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
</tr>
<tr>
<td>Optical density</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to max (sec)</td>
<td>294.3</td>
<td>140.0</td>
<td>296.0</td>
</tr>
<tr>
<td>± 15.5</td>
<td>± 29.0</td>
<td></td>
<td>± 15.4</td>
</tr>
<tr>
<td>Max extent (mV)</td>
<td>16.8</td>
<td>5.0</td>
<td>17.4</td>
</tr>
<tr>
<td>± 2.6</td>
<td>± 1.3</td>
<td></td>
<td>± 1.8</td>
</tr>
<tr>
<td>Release reaction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max extent (μmol ATP/liter)</td>
<td>4.70</td>
<td>1.88</td>
<td>5.87</td>
</tr>
<tr>
<td>± 0.57</td>
<td>± 0.48</td>
<td></td>
<td>± 0.69</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SEM.
CI = confidence interval.
<sup>a</sup>p values based on t test.

Exercise intervention. Diminished secretory response of platelets after activation may indicate increased activity of adenylate cyclase due to stimulation by prostacyclin, the potent antiaggregatory agent. Increased plasma concentrations of prostacyclin have been found in healthy middle-aged men a few days after stopping the exercise training. Furthermore, after a marathon, the subject's platelets are more sensitive to prostacyclin, probably because of increased platelet membrane adenylate cyclase activity. In this study, the change in serum thromboxane was more strongly related to the change in platelet aggregation in the exercise group than in the reference group, which confirms our previous observation on the serum thromboxane-lowering effect of regular mild-intensity exercise.

It is possible that the observation of diminished platelet aggregability represents a response of the ADP receptors of the platelets, but until now no experimental data are available from exercise studies to support this view. Whether platelets respond similarly to other agonists such as collagen and epinephrine remains an open but important question for further studies.

Our findings are not likely to have been influenced by intake of analgesics (which one could expect to be more common in the exercise group), since serum determination for salicylates performed at the end of intervention was negative in all subjects. At the time this study was carried out, salicylates and paracetamol were the only analgesics available without prescription. Furthermore, from the very beginning the subjects were advised to seek help for any medical questions from the research team. The reference group also showed changes in the aggregation measures. Seasonal variations in dietary habits may have contributed. Indeed, we found a constant inverse association between changes in the dietary P/S ratio and the measures of secondary aggregation. Other likely explanations could be biological fluctuation in aggregation phenomena and regression toward the mean. The decisive observation, however, is the net effect attributable to physical training. These data emphasize the importance of having a randomized design with a sedentary control group. Without the reference group, the effect of physical training on platelet aggregability would have been overestimated.

In conclusion, our results demonstrate the role of moderate-intensity regular physical exercise in reducing platelet aggregability, which is observable at least 1 week after discontinuing the exercise. This effect might have a favorable influence on prevention of atherosclerosis and ischemic heart disease.

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