Effect of Physical Fitness on the Coagulant Activity of Healthy Young Men

Z. Rezacian, G. Torkaman, F. Nad-ali, R. Ravanbod,
A.A. Pourfathollah, B. Gousheh, M. Nejatian and M.A. Broumand

Department of Physical Therapy, Tarbiat Modares University, Tehran, Iran
Department of Hematology, Esfahan University of Medical Sciences, Esfahan, Iran
Department of Hematology, Tarbiat Modares University, Tehran, Iran
Behzisty and Rehabilitation Sciences University, Tehran, Iran
Cardiac Rehabilitation Unit, Tehran Heart Center, Tehran, Iran
Department of Pathology, Tehran Medical Sciences University, Tehran, Iran

Abstract: Effects of exercise on different body systems, especially cardiovascular and musculoskeletal systems have been proven. In the haemostatic system the influence of training has been extensively studied but there are a few investigations that analyze the effects of training programs on coagulation factors. In this study we have surveyed the effect of physical fitness on coagulation factors in healthy young men. Subjects were 26 young men without any history of cardiac, coagulation or respiratory problems in themselves or their immediate family, a cardiologist confirmed their cardiovascular health. These men were divided in two groups; the physically active group (Ac) and the sedentary group (Se). The groups were similar in life style except their activity. The physically active men (Ac; n=10) were involved in regular exercise at least three times a week during the last six months. The sedentary men (Se; n = 16) did not participate in any sport activity. According to preliminary clinical examinations, anthropometric variables had no significant differences in either group, but the Bruce test and a standardized ergometry test proved that functional capacity of cardiovascular system in the Ac group is significantly higher than the Se group. Blood data analysis showed that the basal levels of FVIII:c and FIX:c were significantly higher in the Ac group. FVIII:c, FIX:c, fibrinogen and vWF:ag increased in response to exercise while vWF:ag, FVII:c and aPTT decreased significantly. All of these parameters, except fibrinogen, FVIII:c and vWF:ag, returned to resting values during passive recovery. In the Se group elevation in vWF:ag during active recovery, reduction of aPTT and elevation of FVIII:c and FIX:c during passive recovery was statistically significant. Active and sedentary young men are different in resting values of some coagulation factors and their coagulation markers also vary in response to a submaximal exercise program on cycle ergometer. This is due to the difference between physical fitness levels of these two groups. Considering the unfavorable effects of imbalance between coagulation and fibrinolysis, it seems in any type of physical activity especially sport activities the haemostatic balance should be evaluated. Controlling the markers of these systems will help us improve safety of patients in whom exercise is a part of their rehabilitation program, this way it will be possible to enhance positive effects and reduce the possible risks of professional sports for healthy individuals.

Key words: Physical fitness-cycle ergometer-coagulation-FVIII-submaximal exercise

INTRODUCTION

Effects of exercise on different body systems, especially cardiovascular and musculoskeletal systems have been proven and exercise tests are used to diagnosis different cardiovascular and pulmonary diseases (James et al., 1980). Increased understanding of thrombotic mechanisms enables clinicians to predict risk more accurately and increase the scope for target prevention (Laflan and Tuddenham, 1998).

Haemostasis and its exercise-induced changes have been studied by many investigators. Industrialization in developing countries has led to a sedentary lifestyle and ultimately a rise in cardiovascular disorders (Ikaragi et al., 1999). In most of these cases unwanted clot formation is the main etiology whereas in others bleeding is the problem. Although there is controversy in parameters, regular physical exercise programs are the main part of long-term treatment in many of these diseases, including Myocardial Infarction (MI) and Cardiac Vascular Accident (CVA) (Ikaragi et al., 1999, Van Den Burg et al., 1997; Lin et al., 1999, Rankinen et al., 1995; Möckel et al., 2001; Prisco et al., 1998; Wang et al., 1995 and Harsen et al., 1990). For bleeding disorders such as
hemophilia and von Willebrand disease there is great controversy to recommend physical exercise even as recreational activity (Buzzaard, 1997; Koch et al., 1984; Buzzaard, 1996). Recently the World Health Organization (WHO) announced physical inactivity as an independent risk factor for many cardiovascular disorders because of its negative effects on blood pressure, serum lipoproteins and metabolism of carbohydrates. On the other hand regular exercise has been proven to prove positive effects on cholesterol metabolism and regulation of blood pressure (Van Den Burg, 1997). Impact of exercise and training on the haemostasis has been studied extensively; most researchers have analyzed exercise-induced changes in fibrinolytic factors in physically active subjects. Participants usually are athletes involved in endurance exercise such as marathon, long distance running triathlon (Prisco et al., 1998; Bärth et al., 1990) or people who run a distance of at least 20 km recreationally every week (Rock et al., 1997). Unfortunately in sport settings coagulation parameters are recognized much, sometimes general parameters such as fibrinogen, aPTT and FVII-c are analyzed but even then there is usually no control group of sedentary matched participants, to account for the difference.

Six to Seventeen percent of Cardiac Sudden Deaths (CSDs) are related to exercise, i.e., occurring during or a little after physical activity; some of victims are professional athletes who have been involved in sports for a long time. Physical activity stimulates and at the same time prevents unwanted blood coagulation (Albert, 2000), therefore it is very important to study haemostasis and blood coagulation in both physically active and sedentary people. We have studied the effect physical fitness on the level of coagulation factors, especially FVIII.

**MATERIALS AND METHODS**

**Participants:** Subjects were 26 healthy nonsmoker men between 20 and 36 years old. None of them has any history of cardiac, coagulation or respiratory problem in themselves or their immediate family; this was confirmed by a cardiologist. They were divided into an active group (Ac; n = 10) and a sedentary group (Se; n = 16). They were characterized as sedentary if they had a sedentary job and did not participate in any form of sport during their leisure time in the previous year. The physically active groups were participants who used to exercise at least three times a week during the last six months. The study was approved by the Ethical Commission of Tabriz Modares University Medical School and participants joined the study after they filled a written informed consent.

**Bruce test:** Bruce test as a confirmation for participant’s cardiovascular health was conducted under supervision of a heart specialist. The test was discontinued if the following were seen:

- Abnormal Electrocardiograph (ECG)
- Stable Heart Rate (HR) with progression of test stages
- Excessive exhaustion
- HR = 90% Max HR (Max HR = 220 age)

**Assessment design:** To determine functional capacity of participant’s cardiovascular system, a Bruce test was repeated 2 days before Ergometric test between 4:00 and 8:00 P.M. HR and Blood Pressure (BP) were recorded at the last moment of each test and after three passive recovery stages in supine position. Maximal slope (%), maximal speed (km h⁻¹) and energy consumption (METs) were recorded too.

**Ergometric test:** This standardized test, was designed for collection of blood samples at rest, after submaximal exercise and recovery, scheduled between 8:00 and 12:00 A.M. Participants abstained from exercise and heavy physical activity for 24 h and drinking tea or coffee for 12 h before the test (Mittleman, 1993). They were also required to have regular sleep on the previous night and a light breakfast without fat or sugar, on the test day. During the test day each participant came to Tehran Heart Center’s Physiotherapy ward an hour sooner. To reduce the anxiety and to adapt with the test environment they sat down for a while and then took part in the anthropometric assessment. Height, weight and body fat percent were measured.

**Body fat percent:** Triceps, subscapular and chest skin folds were measured by a caliper (Huscoo/ditrite-meter-Pondenral-Nederland b.v) 3 times. The average of each skin fold (mm) was recorded and their sum was transferred in a nomogram to find the corresponding body fat percent (Pollock and Wilmore, 1990).

**Ergometric test:** This test was performed on a cycle ergometer (Bikerace HC600 - TechnOGYM) and comprised five consecutive periods:

- One minute pedaling with workload of 0 and self selected RPM as warm up
- Five minutes pedaling with progressive workload and RPM between 60 and 70 to reach target HR (target HR defined as 70% of maximal HR)
- Ten minutes pedaling with controlled workload and RPM between 60 and 70 to maintain target HR.
Eight minutes pedaling with workload of 0 and self selected RPM as active recovery

Forty five minutes sitting as passive recovery

HR was checked continuously by a Sportester (Polar Heart Rate Monitor 54000144). HR and BP was recorded on the participant’s entrance in the ward, after sitting on the cycle and at the last minute of each step. At the last minute of second and third steps, workload (Watt), distance (km) and energy consumption (METs) were recorded.

Blood collection procedures: The study conducted in Tehran Heart Center (May-July). Three Blood samples were taken from each participant at rest and at the last minute of forth and fifth step of Ergometry test. Blood samples were collected in Nunc 15 ml plastic tubes containing 450 μL of 3.2% trisodium citrate solution. These tubes and the plastic tube for serum had special codes (double blind study). The first sample was drawn from left cubital vein in supine position. The next samples were taken alternatively from participant’s upper extremities. If the cubital vein on one side was not accessible, all samples were taken from the other side. The total volume of blood and sodium citrate was 5 mL. Plasma was separated by centrifugation at 2000 g for 15 min, half of it was frozen immediately and the other half was centrifuged again at 4°C to determine the activity of FVII and vWF. After two days, the prepared plasma samples were transferred to Iranian Blood Transfusion Organization’s coagulation lab.

Statistical analysis: According to Kolmogorov-Smirnov Z-test, all variables in this study had normal distribution. Statistical analyses were performed with SPSS version 11. Differences between Ac and Se groups were calculated by independent t-test. The level of significance was set at p<0.05.

RESULTS

Anthropometric data: There was no significant difference between two groups regarding anthropometric variables (Table 1).

Bruce test results: No significant difference was detected between groups according to Bruce test (Table 2) unless test duration that was significantly longer in Ac group (p = 0.023).

Ergometric test results: Resting HR and the HR before ergometry i.e., when each participant sat on cycle ergometer (HR0), were significantly higher in Se group (p = 0.006 and p = 0.041, respectively). Diastolic blood pressure of Ac group in second and third stages was significantly less than Se group (p = 0.017 and p = 0.038, respectively). Level and workload to reach target HR and maintain it for 10 min, were significantly higher in Ac group (Table 3).

Blood analysis: Analysis of resting value of blood variables showed that resting FVIII:c and FIX:c is significantly higher in Se group (p = 0.033 and p = 0.002, respectively). Table 4 presents blood response to exercise in Ac and Se groups.

- FVIII:c increased during active recovery in both groups but the increase was significant only in Ac group (p = 0.038). In Ac group this increase continued during passive recovery, therefore FVIII:c was 15.6 percent more than its resting level in Ac group at the end of passive recovery (p = 0.007). In Se group, a sharp decrease was detected in FVIII:c during passive recovery (p = 0.027).
- FVIII:c significantly decreased in Ac group during active recovery (p = 0.006), which returned to resting value within 45 min. FVII:c response had a completely contrary pattern in Se group and it decreased considerably during passive recovery (p = 0.052). On the other hand FVII:c was significantly lower in Ac group at the end of active recovery (p = 0.002).
Table 4: Blood variables in Ac and Se groups

<table>
<thead>
<tr>
<th></th>
<th>Ac group</th>
<th>Se group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At rest</td>
<td>Active recovery</td>
</tr>
<tr>
<td>FVIII:c (%)</td>
<td>67.30±0.71±5.3*</td>
<td>76.30±17.6±7.9*</td>
</tr>
<tr>
<td>FIX:c (%)</td>
<td>92.30±12.52</td>
<td>81.40±12.81*</td>
</tr>
<tr>
<td>vWF:ag (%)</td>
<td>129.0±40.35</td>
<td>98.9±29.2±7.9*</td>
</tr>
<tr>
<td>vWF:act (%)</td>
<td>88.40±21.01</td>
<td>108.40±26.51*</td>
</tr>
<tr>
<td>Fibrinogen (mg dL⁻¹)</td>
<td>250.50±40.16</td>
<td>277.80±28.9±7.9*</td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>36.5±2.19</td>
<td>36.6±1.39*</td>
</tr>
<tr>
<td>PT (s)</td>
<td>13.9±1.07</td>
<td>13.5±0.67</td>
</tr>
</tbody>
</table>

*Significant difference between groups. #Significant changes within group regarding its own resting value.

- FIX: c in Ac group increased significantly during active recovery (p = 0.001) and then returned to its basic value. In contrast, FIX:c elevation in Se group was not significant during active recovery however, it decrease to a lower level compared to its basic value during passive recovery (p = 0.005).
- vWF: ag significant increase (p = 0.003) returned to its basic value within 45 min in Se group. In Ac group, its significant decrease during active recovery (p = 0.000) continued during passive recovery (p = 0.000).
- vWF: ac did not change significantly in Se group whereas in Ac group its significant increase during active recovery (p = 0.03) led to a significant difference between the two groups (p = 0.021). During passive recovery it decreased therefore its difference with resting value was near significant (p = 0.058) but it already has a significant difference with vWF:ac of Se group (p = 0.032).
- Fibrinogen In contrast to Se group, increased Fibrinogen in Ac group (p = 0.009) and stayed high during passive recovery (p = 0.031).
- aPTT significant changes were only seen in Ac group. aPTT decreased significantly during active recovery (p = 0.001) and it became less than the aPTT of Se group during active recovery (p = 0.014). aPTT returned to resting value after 45 min.
- PT did not change significantly in either group.

**DISCUSSION**

The goal of the present study was to determine the effect of physical fitness on coagulation system. Preliminary results show that anthropometric parameters including weight, Body Mass Index (BMI) and body fat percent were not significantly different between the two groups. Variables analyzed in the present study can be divided into two main groups: blood variables, which are indicators of blood coagulation activity and physical variables, which are indicators of cardiovascular capacity.

**Physical variables**: The most important physical parameter is resting HR. Resting HR of Ac group was significantly less. Lower resting HR is one of characteristics of aerobic conditioning (Fletcher, 2001; Sugawara, 2001; Braun, 1991). In other words, aerobic conditioning improves myocardial contraction force therefore heart pumps more blood to arteries during each beat and stroke volume increases. As a result, the number of heartbeats required for circulating blood in vascular network decreases, it is the basic difference between athletes and sedentary people.

On the other hand required workload, to reach target HR and maintain it during ergometric test, was significantly higher in Ac group, which is another example of difference in cardiovascular system efficiency of the groups and is a confirmation for higher physical fitness level in Ac group.

According to Table 2, maximal speed and slope of treadmill during Bruce test were not significantly different in the groups whereas test duration was significantly longer in Ac group. In fact, with a same workload progression pattern in order to reach the same HR, Ac group had to exercise for more time than Se group. In the other words, in the same HR, exercise intensity was higher in Ac group. The same phenomena were recorded during ergometric test.

**Blood variables**: Basic value of FVIII:c and FIX:c was significantly higher in Ac group. Other researchers except Van Den Burg et al. (1997) have not compared these values in their studies. They indicated that after an aerobic training program resting FVIII:c increased and aPTT decreased significantly in young healthy men whereas resting FIX:c did not change. The reason for this has not been explained.

This study indicates that a single round of ergometric submaximal exercise increases FVIII:c and FIX:c and decreases aPTT in young healthy men. It is in agreement with what Chicasaro et al. (1995) have found. FVIII and FIX are part of the intrinsic coagulation pathway and aPTT evaluates this pathway therefore, increase in FVIII:c
and FIXc at the same time would lower the aPTT. These changes were statistically significant only in Ac group. Considering this, it seems that these significant changes are exercise-induced. Resting aPTT was not significantly different between two groups.

Because FVIII and FIX response pattern to exercise was the same, the mechanism of this response is also the same. Three mechanisms are involved in exercise-induced release of a factor: synthesis, release from stores and activation of the already inactive factor (Pollock and Wilmore, 1990; Smith, 2003). Exercise stimulates β-adrenergic receptors and increases thrombin formation and thrombin probably increases FVIII in plasma (El-Sayed et al., 2000). Exercise-induced increase in FVIIIc is sometimes due to FVIII release from the storage sites, it can also be due to FVIII activation by thrombin, where FVIII activity ratio increases (Kopitsky et al., 1983).

Kopitsky et al. (1983) showed that exercise enhanced the activity of FVIII in presence of thrombin and concluded that the activation of FVIII is the underlying mechanism of exercise-induced increase in FVIIIc. The disproportional change of FVIIIc and FVIII:ag confirms his theory because he found that FVIIIc elevation was more intense than FVIII:ag Stibbe (1977) and Small et al. (1984) got the same results and therefore, FVIIIc increase in this study is likely due to activation of inactive substrates. In this study the plasma FVIII:ag was not measured, therefore FVIII elevation could also be due to its release from the storage sites.

According to the literature, β-blockers are involved in exercise-induced elevation of FVIIIc (Ingram et al., 1977). Jilma et al. believe that β-adrenergic receptors act via Nitric Oxide (NO), they showed that inhibition of NO production suppresses the enhancement of FVIIIc and vWF:ag considerably. It seems that β-blockers change the mechanism of exercise-induced factor elevation, because taking these drugs for long periods, has no effect on resting values of these factors (Small et al., 1984).

In Ac group, FVIIIc remained high during active recovery whereas in Se group it decreased to under basal levels. This can be due to haemostasis system adaptations in Ac group, which causes sharp and steady elevations of FVIIIc.

FIXc decreased during passive recovery in both groups and in the Se group it was significantly lower than the resting value. The similar pattern of FVIIIc and FIXc response to exercise implies that the immediate response of these factors is the same; in fact physical fitness affects the clearance of active factors and their return to basal values in blood stream. During 45 min of passive recovery, aPTT increased and returned to resting value in Ac group. Therefore, in contrast to FVIIIc, which was easily affected by individuals’ level of fitness, aPTT and FIXc were mainly affected by the nature of the activity rather than the physiological changes induced by systematic physical activity.

In human blood stream, FVIII/vWF is a complex glycoprotein, which leads to clot formation via two different pathways:

- Coagulant activity of FVIII, which increases FX activation rate via activated FIX (IXa). This reaction is performed in presence of phospholipids and Ca²⁺.
- vWF activity, which leads to adhesion of platelets to nonendothelial surfaces. Qualitative or quantitative deficiency of vWF leads to von willebrand disease (Kopitsky et al., 1983).

It seems that physical fitness has positive effects on vWF:ac which in turn regulates FVIII activity (Van Den Berg et al., 1995) therefore vWF response to exercises of various intensity and duration and different training programs seems to be essential.

The patterns of change in vWF:ac and vWF:ag in Ac group were the opposite, vWF:ag decreased significantly and remained low during passive recovery while vWF:ac increased significantly.

Coagulation factors exist in active and inactive forms in plasma. To measure the inactive form the antigen is measured whereas activity is assessed through measuring the biological function of that factor. Only the active forms are important. Activation of a factor’s antigen leads to an increase in the active factor. The active factor is used in its biological pathway and unless it’s replenished from storage sites, it will ultimately decrease. It seems changes in haemostasis system, due to regular exercise, enhance the activation of vWF antigen but does not affect its release into blood stream. In contrast in sedentary people, exercise increases the rate of vWF release into blood stream but does not stimulate its activation therefore, vWF:ag increases without altering vWF:ac levels.

In Se group, vWF:ag increased significantly, however it returned to resting value during passive recovery. The Speiser and Jilma study showed that vWF:ag concentration in sedentary people is not elevated immediately after exercise, but reaches maximal levels 60 min post exercise (Jilma et al., 1997). Here two points are of great importance: 1) the ergometric test in the present study was a submaximal one, changes in coagulation balance are usually related to strenuous exercise; in other words, strenuous exercise unlike moderate exercise enhances fibrinolytic potential in young healthy men. 2) Exercise method is a very important factor,
for example running on treadmill enhances thrombosis far more than ergometer exercise because it leads to more tissue damage. Perhaps this is why our results are so different.

FVII is involved in coronary artery disease. It is a part of the extrinsic coagulation pathway and activates coagulation cascade by binding to tissue factor. Tissue factor releases from damaged atheromatac plaques (Koeing and Ernst, 2000; El-Sayed, 1996). Eating fatty foods increases FVII:c regardless of the type of fat (Mutanen and Freese, 2001) therefore participants abstained from eating fatty foods for several hours before sampling.

Van Den Burg et al. (1995) found that resting FVII:c of young sedentary men (20-30 years old) is lower than the FVII:c of elder sedentary men (35-45 and 50-60 years old). This difference was maintained during submaximal and maximal exercise on cycle ergometer. Also FVII:c did not change during maximal and submaximal ergometric exercise (Van Den Berg et al., 1995). In this study we had the same results. They also showed that by correcting FVII:c for plasma volume, FVII:c decreased in two younger groups (20-45 years old) in response to exercise intensity whereas in the elder group no significant change was recorded. FVII decreases because it binds to thromboplastin and it is proteolysed by elastase, released from active granulocytes (Van Den Berg et al., 1995).

Changes of plasma volume were not measured in this study and the results of FVII analysis are doubttable, although plasma volume alterations during aerobic exercise and submaximal exercise are insignificant and plasma returns to its primary volume within 30 min of passive recovery (Van Den Burg et al., 1997; Kopitsky et al., 1983; Molz et al., 1993; Heilberg et al., 2002). Significant reduction of FVII:c in Ac group implies that the intensity of FVII:c response to exercise was significantly more than the Se group.

Weiss et al. (2002) showed that strenuous long duration exercise (1 h duration) alters blood coagulation via a pathway other than the tissue factor pathway.

Hansen et al. (1990) believed that FVII reduction in strenuous exercise is due to exercise-induced vascular changes. They suggested that FVII binding to thromboplastin or its degradation by proteases such as elastase, is probably the main underlying mechanism. They also believed that Prothrombin Time (PT) is not sensitive enough to measure changes in extrinsic pathway because it did not change in their study (Hansen et al., 1990). On the contrary Heilberg et al found that PT increased during submaximal exercise in diabetics and healthy people.

In the present study PT did not change in either group. This was in agreement with other studies (Hansen et al., 1990; Chicharro et al., 1995) although Van Den Burg et al. (1995) found that submaximal exercise in individuals over 20 years old reduces PT significantly. This reduction was more pronounced in maximal exercise and was maintained during passive recovery (Van Den Berg et al., 1995). Ferguson et al. (1987) also showed that strenuous exercise reduced PT significantly in active men but it returned to basal levels after an h.

Fibrinogen is the best coagulation marker in evaluating the risk of coronary artery diseases. Its plasma concentration is considerably influenced by the quality and quantity of dietary fats (Mutanen and Freese, 2001). If plasma fibrinogen concentration is increased to above 3 g L⁻¹, the risk of cardiovascular diseases duplicates (Koeing and Ernst, 2000). Fibrinogen is the final substrate in the coagulation cascade converted to fibrin by thrombin. This process depends on the concentration of fibrinogen in plasma. In addition, fibrinogen is important in the primary stages of atherosclerotic plaque formation. There is a great controversy about the effects of strenuous exercise on fibrinogen; some authors believe it dose not change and some find that it decreases (Smith, 2003; El-Sayed et al., 2000), however it seems that if plasma volume changes were taken into account, fibrinogen reduction truly occurs (Smith, 2003).

In the Ac group, fibrinogen increased significantly during the last session and stayed high for 45 min. In the Se group, the same pattern of changes was detected but it was not significant. This confirms the positive effects of training on exercise-induced changes of blood viscosity.

According to Berg et al. (2002) exercise led to a small but significant increase in fibrinogen in healthy women and MI patients, they believed aspirin was the main factor in increasing fibrinogen. In the present study, we had the same results in healthy men. The fibrinogen change is apparently intrinsically induced rather than pharmacologically.

It seems that physical fitness directly influences the pattern of coagulation system changes. It should be considered when exercise programs are recommended for cardiac patients. At the same time, it implies that physical activity could be useful for patients who suffer from coagulation deficiencies such as hemophilia (Ravanbod et al., 2003) and von willebrand disease.

In conclusion, a single round of submaximal exercise on cycle ergometer, optimizes the coagulation balance more in the physically active healthy young men compared to the sedentary group.

2037
ACKNOWLEDGMENT

We thank all of our coworkers who participated in this study. This study supported by Tarbiat Modares University, Iranian Blood Transfusion Organization and Tehran Heart Center.

REFERENCES


