Exercise and Training Effects on Blood Haemostasis in Health and Disease
An Update

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Abstract

In recent years, the dysfunction of the haemostatic system in relation to the clinical complications from arteriosclerotic and cardiovascular diseases has become more recognised. Blood coagulation and fibrinolysis comprise two important physiological systems, which are regulated by a balance between activators and inhibitors. Activation of blood coagulation is associated with accelerated clot formation, whereas activation of blood fibrinolysis enhances the breakdown of the blood clot. Available evidence suggests that strenuous exercise induces activation of blood coagulation with simultaneous enhancement of blood fibrinolysis. Although the responses of blood coagulation and fibrinolysis appear to be related to the exercise intensity and its duration, recent reports suggest that moderate exercise intensity is followed by activation of blood fibrinolysis without...
concomitant hyper-coagulability, while very intense exercise is associated with concurrent activation of blood coagulation and fibrinolysis. Similar to blood coagulation and fibrinolysis, systemic platelet-related thrombogenic factors have been shown to be involved in the initiation and progression of atherogenesis and plaque growth. Although exercise effects on platelet aggregation and function in healthy individuals have been examined, the results reported have been conflicting. However, for patients with coronary heart disease, the balance of evidence available would strongly suggest that platelet aggregation and functions are increased with exercise. Few studies are available concerning the influence of training on blood coagulation and fibrinolysis and the exact effects of exercise training on the equilibrium between blood coagulation and fibrinolysis is not as yet known. Although the effects of physical training on platelets have been briefly investigated, available meagre evidence suggests that exercise training is associated with favourable effects on platelet aggregation and activation in both men and women.

1. An Overview of Blood Haemostasis

Blood haemostasis represents a complex interaction between the coagulation and fibrinolysis systems, platelets and other circulating cells and the vascular wall. Blood coagulation is an important mechanism in the haemostatic system that involves a complex series of interactions between proteases, enzymes, and co-factors that lead to the generation of thrombin and the formation of the fibrin-rich clot. Fibrin-rich clot formed at the end of the blood coagulation cascade, plays a temporary role and must be removed when normal tissue structure and functions are restored. Blood coagulation can be subdivided into three main pathways: (i) the intrinsic factor X activation pathway; (ii) the extrinsic factor X activation pathway; and (iii) the common pathway, which is the sequence of events following the activation of factor X up to the formation of the fibrin clot.\(^1\) The fibrinolytic system is the main mechanism designed for the clot removal and controls the enzymatic degradation of fibrin. The dominant mechanism for fibrinolysis in vivo is the plasminogen-plasmin system, which can be activated by intrinsic and extrinsic pathways.\(^2\) The key enzyme, directed at the dissolution of fibrin, is plasmin. Plasmin circulates in the blood in an inert form, plasminogen, which can be converted to the active enzyme plasmin by plasminogen activators. The exposition of certain epitopes on the fibrin molecule triggers the activation of plasminogen to plasmin by plasminogen activators. Platelets play a major role in the blood-clotting process, particularly in the intrinsic pathway of blood coagulation cascade. The role of the platelets in the formation of the haemostatic plug implies that adhesion and aggregation are initial and mandatory functions. The other important platelet functions are transport of fibrinogen and other compounds, release of various substances during aggregation (e.g. β thromboglobulin, platelet factor IV) and de-aggregation of previously aggregated platelets.\(^3\) This review updates and critically examines the published work on the effects of exercise and training on the main systems of blood haemostasis, namely blood coagulation, blood fibrinolysis and platelets.

2. Blood Coagulation

Activation of blood coagulation results in the formation of thrombin and ultimately the formation of fibrin. The key function of thrombin is the catalytic conversion of soluble fibrinogen to insoluble fibrin.\(^4\) Thrombin also activates platelets and several other proteins in the coagulation cascade leading to enhanced thrombin generation and clot formation. Although the intrinsic and extrinsic pathways of the coagulation system lead to the formation of a fibrin clot, the main pathway of coagulation activa-
Intrinsic pathway
- Surface
- HK
- PK
- XII
- XIIa
- Ca2+
- HK
- Ca2+
- PL

Extrinsic pathway
- Vascular injury
- Ca2+
- XI
- XIa
- IX
- IXa
- Ca2+
- PL
- VII
- VIIa + TF

Fig. 1. Blood coagulation pathways. HK = high molecular kininogen; PK = prekallikrein; PL = platelets; TF = tissue factor.

ion is the extrinsic route. In physiological situations, the intrinsic pathway seems only to have a booster effect. The intrinsic cascade is initiated when contact is made between blood and exposed endothelial cell surfaces. The extrinsic pathway is initiated upon vascular injury, which leads to exposure of tissue factor. Although they are initiated by different mechanisms, the two unite on a common pathway that leads to blood clot formation. Both pathways are complex and involve numerous different proteins termed ‘clotting factors’. The blood coagulation pathways are illustrated in figure 1.

2.1 Acute Exercise and Blood Coagulation

2.1.1 Studies in Normal Healthy Individuals

It has long been known that blood is hypercoagulable following strenuous exercise. Exercise-induced shortening of whole blood clotting times and activated partial thromboplastin time (aPTT) is well documented.[5-11] However, results reported on prothrombin time (PT) and thrombin time (TT) in response to exercise have been controversial.[8,11-13] Research has shown both a significant shortening[12] and no significant difference,[8,11,13] in PT following exercise. El-Sayed and Davies[8] have demonstrated that exercise significantly shortens TT. Changes in aPTT and PT persist from 1 to 24 hours post-exercise.[5,13] Although the reasons for the conflicting results of PT and TT with exercise are not known, this could be attributed to the use of different exercise protocols with varying intensity in different study populations (patients, normal sedentary individuals, habitual exercisers and athletes) with a wide range of physical ability and cardiorespiratory fitness. In addition, PT and TT are non-specific overall in vitro measures of blood coagulability, which are affected by a host of factors including natural coagulation inhibitors. Exercise-related hypercoagulability is mainly due to an increase in coagulation factor VIII (FVIII)[5,8,14] with no parallel alterations of other clotting factors. Exercise bouts of varied intensity and duration have all induced significant increases in FVIII coagulant ac-
tivity.\cite{5,8,14} It has been demonstrated in earlier studies that the increase in FVIII coagulant activity and antigen was positively associated with endurance exercise intensity,\cite{15} and persists during recovery.\cite{5,14} Similar to endurance exercise, FVIII activity rises following resistance exercise, and appears to be positively correlated with the volume of weight lifted.\cite{16} The mechanism by which exercise increases FVIII is not fully understood. Earlier evidence suggests that the increase in FVIII may either be due to activation within the circulation, or to the release of stored or freshly synthesised FVIII.\cite{16} In vitro exposure of FVIII to catalytic concentrations of thrombin lead to a significant increase in FVIII,\cite{17} thus suggesting that this increase might also be associated with thrombin formation. The \(\beta\)-adrenergic pathway has been implicated as a possible pathway mediating an exercise-induced increase in FVIII.\cite{18} The study of Jilma et al. showed that partial blockade of nitric oxide synthase with L-NMMA blunts the exercise-induced increase in von Willebrand factor antigen and FVIII coagulant activity and that nitric oxide generation plays a role in the \(\beta\)-adrenoreceptor stimulation.\cite{19}

Although exercise-induced FVIII increase and a shortening of the aPTT following exercise is well documented, it is doubtful if this mirrors a hypercoagulable state in vivo. It may be that the shortening of aPPT merely reflects a pre-start phase but not definitive activation in blood coagulation. As thrombin plays a central role in the blood coagulation cascade, its generation or the enhanced potential of its generation must exist in the hypercoagulable state. However, results reported on effects of exercise on markers of thrombin generation are conflicting. For example, changes in molecular markers of thrombin formation and fibrin generation were more pronounced after running compared with swimming and cycling.\cite{20} The authors also demonstrated that only the running exercise was associated with a significant increase in thrombomodulin, thus suggesting that mechanical factors and endothelial cell activation might be involved in exercise-induced hyper-coagulability. In contrast, Hilberg et al.,\cite{21} showed no significant differences between blood coagulation responses to exhaustive treadmill or cycle exercise. Shortening of aPTT and increasing of F1+2 indicated an activation of the coagulation system during exhaustive treadmill and cycle exercise. However, unchanged intrinsic and extrinsic endogenous thrombin potential with exercise led to the conclusion that the potential for thrombin generation in healthy young male study participants is insignificant.\cite{22} It may also be argued that thrombin, once generated in response to strenuous exercise, is inactivated by antithrombin and does not give rise to marked fibrin formation.

### 2.1.2 Studies in Patient Populations

Earlier studies in male patients with coronary artery disease and healthy individuals\cite{23} also indicated that a single bout of rehabilitative exercise is associated with simultaneous activation of blood coagulation and acceleration of blood fibrinolysis. It should be noted that this occurred with very small thrombin formation and fibrin generation. Comparison between normal healthy individuals and patients with peripheral arterial occlusive disease showed higher d-dimer levels both at rest and after exercise in the patient group. In addition, submaximal exercise led to increased thrombin generation in the patient group, whereas similar exercise intensity and duration failed to induce a similar response in the control individuals.\cite{23} In contrast, Eriksson-Berg et al.,\cite{24} demonstrated that middle-aged women with previous myocardial infarction had similar coagulation and fibrinolytic responses to submaximal exercise as healthy women. A rise in fibrinogen and von Willebrand factor antigen concentrations were reported in both groups following 30 minutes of submaximal exercise on a bicycle ergometer.\cite{24} However, exercise did not induce thrombin generation and fibrin formation, as assessed by thrombin-antithrombin complex (TAT) and d-dimer. Oral contraceptive use has long been associated with hypercoagulability, and data continue to support an increased risk for thromboembolic disorders among oral contraceptive users, particularly venous thrombosis. Recent data support this concept and further confirmed that oral contraceptive use blunts the
normal fibrinolytic response to exercise along with increased blood coagulability.[25]

2.1.3 Acute Exercise and Blood Coagulation: Implications for Intensity and Duration

Exercise-induced hyper-coagulability as reflected by an increase of FVIII concentration and shortening of activated partial thromboplastin time[1,2] suggests that this activation occurs via the intrinsic, but not the extrinsic, coagulation pathway. The possible involvement of tissue-factor-dependent pathway (extrinsic pathway) in exercise-induced activation of blood coagulation was examined recently.[26] Although a standardised 1 hour of maximal treadmill running was followed by a significant increase in thrombin generation and fibrin formation, there was no exercise-induced increase in tissue factor expression.[26] It is generally accepted that exercise causes an activation of blood coagulation, but it is disputable whether this leads to significant in vivo thrombin generation and fibrin formation. Weiss et al.[27] examined the relationship between exercise intensity and the activation of coagulation and fibrinolysis. They showed that exercise at ~68% maximal oxygen uptake (VO2max) increased plasmin formation without corresponding increases in the markers of blood coagulation activation. However, exercise at ~83% VO2max was associated with an increased plasmin formation, but this occurred in parallel with an increase in markers of blood coagulation. Thus, moderate exercise appears to enhance in vivo blood fibrinolysis, whereas very heavy exercise activates blood fibrinolysis and blood coagulation simultaneously.

Long-term exercise such as marathon running was followed by an activation of blood coagulation, as indicated by the formation of thrombin and cross-linked fibrin.[28] It should be noted, however, that the acceleration of blood coagulation was smaller than the activation of blood fibrinolysis. Patients with peripheral arterial occlusive disease exhibited increased thrombin formation post-submaximal exercise, although no such increase was shown in healthy control individuals.[23] These results would suggest that markers of activation of blood coagulation and indicators of enhanced fibrinolysis are related to exercise intensity and the health of the populations studied. Recently, Gunga et al.[29] examined the responses of markers of coagulation, fibrinolysis and angiogenesis to a strenuous short-term exercise test (Wingate test). These authors demonstrated that PT, FVIII and d-dimer increased during the Wingate test and remained elevated during recovery. In addition, fibrin monomers and tissue-plasminogen activator (t-PA) were approximately 15 and 4 times higher, respectively, immediately post-exercise and remained elevated during the recovery period.

TAT and prothrombin fragments 1+2 (F1+2) have been utilised as markers of blood coagulation activation in exercise. Significantly increased TAT has been observed following long-distance running[6,30] and post-maximal incremental cycling.[31] This coincided with a significant increase in PTF1+2 concentration.[10,30,32,33] In vivo hyper-coagulability may also be linked with the formation of fibrinopeptide A. However, exercise studies on this marker of hyper-coagulability have produced conflicting results. A significant increase of fibrinopeptide A was found following exhaustive exercise,[32,33] although other studies have demonstrated no significant change.[6,10] These discrepancies may be attributed to differences in exercise protocol, training status and the analytical methods employed for the determination of fibrinopeptide A. Therefore, evidence suggesting that acute physical exercise in healthy individuals leads to increased thrombin generation and fibrin formation, in vivo, remains debatable. Mandalaki et al.[34] studied blood coagulation inhibitors and reported a significant decrease in antithrombin-III activity post-marathon run. This finding was confirmed by Huisveld et al.[35] who further reported a reduction in the blood fibrinolysis inhibitors α2-antiplasmin, and C1-inactivator. Other studies have reported no significant change in antithrombin-III concentrations following exhaustive exercise.[6,10,36] The effects of exercise on these markers of blood coagulation and fibrinolysis remains speculative and the evidence available is insufficient to draw a valid conclusion.
2.1.4 Plasma Fibrinogen in Acute Exercise

Fibrinogen is a useful protein that furnishes the foundation of the blood clot, haemostatic plugs and sloughs upon wounds. Fibrinogen can also form dangerous thrombi, which can occlude blood vessels and block oxygen supply. Several prospective epidemiological studies have clearly suggested that elevated plasma fibrinogen concentration represents a major, independent cardiovascular risk factor. Fibrinogen is synthesised by parenchymal liver cells and its plasma degradation products and cytokines, particularly interleukin-1, govern its release into the circulation. Approximately 80–90% of the body’s fibrinogen circulates in blood plasma. Dissolved in the plasma, fibrinogen is transported to any site where it is needed, where inflammatory or healing processes are occurring. The average concentration of plasma fibrinogen in normal healthy individuals ranges from 2 to 4 g/L, with the molecule’s half-life spanning from 3 to 6 days. Fibrinogen promotes platelet aggregation, plays a pivotal role in the final phase of the blood coagulation cascade, and is one of the main determinants of plasma and whole blood viscosity. Plasma fibrinogen concentration is increased with inflammation, in smokers, in obese individuals, and also in patients with diabetes mellitus and abnormal lipid profiles. Fibrinogen is generally considered an important acute phase reactant protein that plays a pivotal role in the blood coagulation cascade, and is one of the main determinants of blood rheology and smooth muscle cell migration and proliferation.

Studies investigating the effects of acute exercise on plasma fibrinogen concentration have produced conflicting data. Some studies have shown that exercise has no significant effects on plasma fibrinogen; others have demonstrated either an increase or decrease following exercise. Bartsch et al., examined plasma fibrinogen in 19 well trained athletes following a 100km race. Although plasma total protein concentration was significantly higher, plasma fibrinogen concentration was significantly lower after the race. Exercise-induced hyperfibrinogenolysis was suggested as a plausible mechanism for the reduction in fibrinogen after the race. It is known that prolonged exercise such as a 100km race is usually associated with an expansion of plasma volume; therefore, it is possible that the decrease in plasma fibrinogen concentration observed in earlier studies by Bartsch et al. may have also been due to prolonged exercise-associated haemodilution. This explanation is based on two observations: (i) post-exercise blood sampling was delayed for between 5 and 53 minutes; and (ii) individuals ingested fluid during the race and this may have induced a hyper-hydration status in the athletes with a resultant dilution effect on plasma fibrinogen. Removal of fibrinogen from plasma by transudation into the interstitial space and increased fibrin clot formation have also been suggested as mediating factors for the reduction in plasma fibrinogen following exercise. Comparable results were reported by Osterud et al. who examined plasma fibrinogen concentration following different exercise protocols in 31 male and female skiers. Although the fall in plasma fibrinogen concentration post-exercise in the latter study is similar to earlier reports, the magnitude of the decrease in fibrinogen concentration was small and might be due to limited hyper-fibrinogenolysis. This explanation is based on the evidence reported by Collen et al. who found hyper-fibrinogenolysis with exercise as reflected by a shortened half-life of radio-labelled fibrinogen.

In contrast, other studies demonstrated an increase in plasma fibrinogen in normal healthy individuals and in patients with pulmonary disease, cardiovascular disease, and with diabetes. It should be noted that when post-exercise plasma fibrinogen concentration was adjusted for plasma volume loss, both multistage maximal treadmill exercise stress test and maximal cycling exercise failed to evoke a significant change in plasma fibrinogen concentration. Therefore, it is unclear whether or not exercise induces an actual increase in plasma fibrinogen concentration, as a consequence of fresh release from the liver and/or an apparent rise due to associated haemoconcentration. This argument is based on the evidence that acute exercise brings about a transfer of the blood’s fluid to and from the circulation, depending on
exercise intensity and duration, and this may affect the relative concentration of plasma fibrinogen. Haemoconcentration usually occurs in response to vigorous exercise, with a linear relationship between the amount of fluid transferred from the vascular tree and exercise intensity. El-Sayed et al., hypothesised that an inability to consider the dynamic nature of plasma volume during exercise in relation to alterations in plasma fibrinogen concentration is one of the main causes of the discrepancy reported in the literature. It was concluded that changes in plasma volume in response to exercise should be taken into account when interpreting exercise effects on plasma fibrinogen concentration. Differences in exercise protocol, training status, health of the individual, and the analytical methods employed for the assessment of plasma fibrinogen are probably responsible for the reported inconsistencies.

2.2 Exercise Training and Blood Coagulation

Little information seems to be available on the effects of exercise training on blood coagulability. Cross-sectional data of PT and APTT as overall measures of blood coagulability showed no difference amongst sedentary individuals, joggers or in marathon runners at rest or post-exercise. These results are in agreement with those reported in athletes and non-athletes who exhibited similar TT at rest. Likewise, a longitudinal study demonstrated no significant change in TT or PT following 3 months of endurance training. When physical activity level was assessed by a questionnaire, a lower APTT, but not TT, was found in active compared with non-active individuals. Physical training in post-myocardial patients seems to suppress blood coagulability because APTT at rest is significantly longer and FVIII activity and antigen were lower after training in these patients.

Resting levels of FVIII activity and FVIII antigen do not change with training in sedentary individuals or in endurance-trained athletes. However, post-myocardial patients lowered their resting levels of FVIII activity and FVIII antigen after 4 weeks of physical training. The normal increase in FVIII activity post-exercise also seems to be unaltered following 12 weeks of standardised aerobic training. To determine the effects of aging and physical conditioning on the blood coagulation mechanism, Van den Burg et al., examined 39 sedentary men of different age groups before and after 12 weeks of endurance training. With the exception of a significant increase in F1+2 in the elderly, reflecting additional thrombin formation, no effect of training on other blood coagulation indices was found on resting or submaximal exercise in any of the groups; thus in agreement with previous longitudinal studies. These meagre results suggest that FVIII activity and FVIII antigen levels at rest or after exercise remain unchanged in response to training in normal healthy individuals, although not in cardiac patients.

The relationship between plasma fibrinogen and exercise training has been previously examined and recently reviewed and will only be briefly discussed here. Epidemiological studies have implicated a favourable association between physical training and plasma fibrinogen concentrations. However, available longitudinal evidence is conflicting with some research suggesting that physical training may reduce plasma fibrinogen concentration in patients and in elderly males, but not in young males. Surprisingly, and in contrast to these results, plasma fibrinogen concentration increased significantly in elderly males post-intensive training, and this coincided with a significant rise in C-reactive protein. It was concluded that vigorous training in elderly males might cause a chronic increase in acute phase reactant proteins such as fibrinogen. Unlike elderly males, recent evidence suggested that the training effects on plasma fibrinogen in elderly females appear to be negligible. In addition, Dunstan et al., suggested that 8 weeks of moderate- or low-intensity exercise programmes had no significant effect on fibrinogen in patients with dyslipidaemic type 2 diabetes compared with control groups. Similarly, exercise training for 8 months had no significant effects on haemostatic markers including plasma fibrinogen in obese individuals. Therefore, no valid conclusion regarding
the effect of training on plasma fibrinogen could be drawn from the above reports and further investigations are required.

3. Blood Fibrinolysis

Blood fibrinolysis is an important physiological mechanism that is designed to remove, through proteolysis by the enzyme plasmin, stable fibrin in a retracted blood clot, i.e. one that has served its original haemostatic function, but is now redundant and requires removal in order to maintain vascular blood flow. Fibrinolysis is initiated by the secretion of plasminogen activators: t-PA and urokinase-plasminogen activator (u-PA). t-PA is secreted by the endothelium of blood vessels and circulates in the blood in an active single-chain form. The activity of t-PA is increased in the presence of fibrin. u-PA is secreted by the kidney and circulates in an inactive single-chain form (scu-PA) that must be converted by plasmin into the active two-chain form. The principal inhibitor of both t-PA and the two-chain u-PA is plasminogen activator inhibitor-1 (PAI-1). The t-PA and two-chain u-PA activate plasminogen to plasmin. The plasmin produced degrades fibrin, in turn producing d-dimer and other fibrin degradation products. The principal inhibitor of plasmin is antiplasmin (figure 2).

3.1 Acute Exercise and Blood Fibrinolysis

It is generally accepted that intense exercise induces significant activation of fibrinolysis as a consequence of t-PA release from the vascular endothelial cells. Evidence is also available to suggest that plasma levels of u-PA increase significantly post-exercise. It should be noted, however, that peak levels of u-PA and t-PA do not coincide in time or magnitude in response to maximal exercise. This may suggest independent mechanisms regulating exercise-induced increases in the level of u-PA and t-PA. Large increases (75–250%) in fibrinolytic activity are not apparent until the heart rate reaches 50% of maximum with the greatest increase occurring between 70% and 90% of maximal workload. Although this hyper-fibrinolysis is transient, reports have been conflicting concerning its return to baseline level post-exercise. With a time course of 45–60 minutes following intense exercise, or 2 hours following long distance running, and 24 hours post-marathon.

Fig. 2. Blood fibrinolysis mechanisms. PAI-1 = plasminogen activator inhibitor-1; PAI-2 = plasminogen activator inhibitor-2; t-PA = tissue-plasminogen activator; u-PA = urokinase-plasminogen activator.
measured by t-PA and PAI-1 activity and antigen levels. However, in response to maximal exercise, atenolol and propranolol attenuated the fibrinolytic response, as evidenced by a lesser increase in t-PA activity and a lesser reduction in PAI-1 activity than responses with placebo. These results contrast with the evidence that during exercise t-PA release occurs before an increase in adrenaline, suggesting that the main release of t-PA is mediated by some other non-adrenergic mechanism, possibly vasopressin.[53] Earlier studies have demonstrated a significant reduction of PAI-1 activity following aerobic and anaerobic exercise.[42,53,71-73] Maximal treadmill exercise in normoxaemic and hypoxaemic conditions significantly decreased PAI-1 activity.[80] Resistance exercise also produced a similar reduction.[74] Other studies have failed to detect any change in PAI-1 following exhaustive aerobic[25] and isometric[75] exercise protocols. As it is the case with t-PA response, the PAI-1 response to exercise is related to the training status of the individual.[73]

Attempts have been made to relate the activation of fibrinolysis with changes in fibrinogen concentration measured in vitro, and with alterations of the markers of fibrinogen and/or fibrin degradation in vivo. Significant increases in fibrin/fibrinogen degradation products (Fb/FgDP) have been demonstrated following various exhaustive exercise protocols.[12,30] The plasma Fb/FgDP response appears to be related to exercise intensity and the training status of the individual.[36,70] An increased level of another in vivo marker of hyper-fibrinolysis, d-dimer, was observed when submaximal exercise was followed by short-term maximal exercise[111] and following endurance exercise.[5,7,13,30] These results suggest that strenuous exercise results in hyper-fibrinolysis in vivo. This is not a uniformly reported finding since other studies[81,82] have failed to demonstrate changes in Fb/FgDP in response to exercise. Therefore, the actual effect of exercise on Fb/FgDP has yet to be resolved.

3.2 Exercise Training and Blood Fibrinolysis

Thrombosis plays significant role in the pathogenesis of acute myocardial infarction, unstable angina and sudden cardiac death.[83] Although the reduction in cardiovascular risk associated with regular physical activity has been repeatedly reported[84-86] the pathway(s) via which this occurs is not fully understood and remains speculative. It is suggested that this may be linked with exercise-induced favourable effects on blood fibrinolysis.[1] However, it is important to note that the effect of physical training on parameters pertinent to blood fibrinolysis have produced inconsistent results. For example, no relationship between physical training status and resting fibrinolytic activity has been reported when blood fibrinolysis was assessed by global methods such as euglobulin clot lysis time and fibrin plate methods.[12,56] However, when more specific techniques were employed, higher resting t-PA activity and t-PA antigen levels were found in inactive compared with active individuals.[70,73] Comparable results were reported in which PAI-1 activity level was decreased following 8 months of training, but this decrease failed to reach the designated level of significance (p > 0.05) due to large group variances and seasonal variations.[87]

Higher PAI-1 values were found in post-myocardial patients when compared with elderly individuals and this was also noted in athletes compared with age-matched sedentary individuals and elderly sportsmen.[49] Evidence is also available to suggest that exercise rehabilitation programmes are associated with significant reductions in PAI-1 level in cardiac patients, but not in healthy controls.[43] Three months of detraining seems to be sufficient to reverse the favourable reduction in PAI-1 activity observed post-training.[88] Two studies on the effect of exercise training on blood fibrinolysis in patients with type 2 diabetes have produced varying results. An increase in the resting level of blood fibrinolysis was demonstrated after training in one study,[89] but not in the other in which blood fibrinolysis was unaltered at rest or in response to exercise.[90]

Enhanced fibrinolysis in response to exercise seems to be related to the training status of the individual.[12] This concept was confirmed[70,73] as higher t-PA release and lower t-PA/PAI-1 complex were found following exercise in physically trained
individuals than in untrained individuals. Although the background of, and the mechanism responsible for, the higher t-PA release in endurance-trained individuals is not fully understood, this could be attributed to an enhanced sensitivity and up-regulation of the endothelial cells to release t-PA. Diminished fibrinolytic activity, due to an increase in PAI-1, is often seen in patients with myocardial ischaemia. However, Estelles et al. showed no significant effect of training on PAI-1 activity in cardiac patients. Likewise, Dunstan et al. showed no significant training effect on PAI-1 antigen in patients with diabetes. More recently, Barbeau et al. examined the effects of exercise training and adiposity on haemostatic parameters in obese youths and demonstrated that 8 months of physical training had no effect on PAI-1, d-dimer and C-reactive protein. It is interesting to note that the individuals who did not participate in the exercise rehabilitation programme, and acted as controls, exhibited increased PAI-1 activity. The increase in PAI-1 activity following training in the control group is intriguing, and the exact mechanism responsible for this was not adequately explained. The discrepancy in the results cited above may be attributed to methodological differences particularly exercise intensity and duration and also the analytical techniques employed for the measurement of PAI-1 activity.

Earlier studies suggested that the favourable effects of training on blood fibrinolysis appear to be age-related, as higher fibrinolytic potential was observed post-training in elderly, but not in young, study participants. For example, elderly individuals exhibited an increase in t-PA and a decrease in PAI-1 activity, and decrease in PAI-1 antigen following different training programmes. Available evidence suggests that physical training can also favourably affect blood fibrinolysis in the young. In contrast, Van den Burg et al. demonstrated no effect of training on resting and submaximal levels of fibrinolytic parameters in sedentary men of different age groups.

4. Blood Platelets

Formation of a stable haemostatic white thrombus is certainly one of the most important functions of the blood platelet. In addition, platelets play a major role in the blood-clotting process. It has been suggested that platelets are important in many of the reactions in the intrinsic pathway of coagulation and plays a pivotal role in arteriole thrombosis. The other important platelet functions are transport of fibrinogen and other compounds, and the release of various substances during aggregation and de-aggregation of previously aggregated platelets. Immediately after vessel damage, platelets adhere to the site of vessel injury. This adhesion is mediated by the binding of platelet receptors to von Willebrand factor and to collagen in the exposed sub-endothelial layer of the vessel wall. This leads to platelet aggregation, which is mediated by binding of the platelet receptor to fibrinogen. Additionally, platelets undergo activation involving significant morphological changes accompanied by exposure of new proteins on the platelet surface and release of contents from alpha granules and dense granules in resting platelets.

4.1 Acute Exercise and Platelet Count

Exercise is usually followed by a significant increase in platelet count. The increase in platelet count has been attributed to the release of platelets from the vascular beds of the spleen, bone marrow, and also from an intra-vascular pool of the pulmonary circulation and the lungs. It is interesting to note that Dawson and Ogston reported a significant rise in platelet count after exercise in splenectomised humans. Adrenaline infusion has been shown to produce powerful contraction of the spleen, where a third of the platelets are stored and this might account for the large rise in circulating platelet numbers in exercise. In addition, an in vivo population produced by fragmentation of trapped megakaryocytes in the bone marrow and lungs, or sequestration from the liver and lungs, may also play a role in exercise-induced thrombocytosis.
4.2 Acute Exercise and Platelet Aggregation and Activation

4.2.1 Studies in Normal Healthy Individuals

In the past two decades, activation of platelets has been linked to cardiovascular complications following strenuous exercise.\[98\] In healthy individuals, investigations of exercise and platelet aggregation and activation have produced conflicting results.\[99\] For example, platelet activation post-exercise is increased in sedentary individuals but not in physically trained individuals.\[100\] P-Selectin expression, using flow cytometric analysis was utilised as a marker of in vivo platelet activation. A significant increase in P-selectin was found following a triathlon race, but the time course of platelet activation did not reach the baseline level until 120 minutes following the triathlon competition.\[101\] In addition, Hilberg et al.\[102\] could show that P-selectin is only minimally enhanced in healthy individuals after a maximal treadmill ramp test. The mechanism by which activated platelets are cleared out of the circulation is not as yet known. Platelets possibly lose surface P-selectin but remain activated in the circulation.\[103\] Mechanical factors and possibly endothelial micro-lesions with collagen exposure have been implicated as possible stimuli responsible for platelet activation during exercise.

Although the effects of endurance exercise on platelet aggregation and function have been previously investigated,\[1\] the influence of resistance exercise has only been recently examined\[104\] using new flow cytometry techniques to evaluate platelet activation by the detection of the surface-expressed alpha granule protein P-selectin. Although platelet count was not altered, a marked increase in P-selectin expression was found following maximal isometric exercise not only in the dominant arm but also the resting contralateral arm.\[104\] The effects of resistance exercise with varying intensity on platelet aggregation and activation in male study participants was recently investigated.\[105\] Resistance exercise was followed by a significant increase in platelet count, plateletcrit and mean platelet volume, but this rise was not related to the exercise intensity. Exercise was also associated with a significant increase in platelet aggregation, but this only occurred with the high but not with the low concentration of adenosine diphosphate (ADP). Beta thromboglobulin (B-TG) is a platelet-specific protein that is released from alpha granules during platelet activation and its concentration in plasma reflects platelet activity. Resistance exercise evoked an increase in B-TG, but this rise only reached the assigned level of significance (p < 0.05) following the 80% one repetition maximum exercise trial.\[105\] This finding may suggest that exercise intensity is an important factor in platelet hyperactivity in vivo. It should be noted that a recent study demonstrated a decrease in B-TG following endurance exercise\[106\] and it remains an open question whether an exercise-induced increase in platelet activation markers such as B-TG and platelet factor IV really occur.\[107\]

Platelet responses to exercise depend on several factors including exercise intensity, exercise duration, and the physical fitness status of the individual. Moderate-intensity exercise suppresses platelet function, while strenuous exercise increases platelet aggregation and activation.\[1\] Recent evidence further indicates that exercise activates platelets in vivo as reflected by an increase in the number of platelet-aggregate platelet aggregates, a rise in P-selectin, and also enhanced platelet sensitivity and aggregation to in vitro stimulation by aggregating agents. However, aspirin (acetylsalicylic acid) administration failed to attenuate the enhanced platelet aggregation in response to exercise, or decrease P-selectin expression or soluble P-selectin.\[108\]

The effects of mental stress, dynamic exercise and adrenaline infusion on platelet sensitivity to thrombin using flow cytometry analysis of platelet fibrinogen binding in whole blood were recently compared. Platelet aggregation was also measured in vitro using filtragometry. Strenuous exercise and adrenaline infusion, but not mental stress, reversibly increased platelet aggregation and platelet sensitivity to thrombin.\[109\] In a similar study,\[110\] the effects of aerobic exercise, epinephrine (adrenaline), norepinephrine (noradrenaline), and the combination of both catecholamines on shear-induced platelet reactivity were examined in normal male study partici-
4.2.2 Studies in Patient Populations

Results on platelet aggregation and activation in response to acute exercise in normal healthy individuals have been conflicting and difficult to interpret. However, for patients with coronary heart disease, the balance of evidence available would strongly suggest that platelet aggregation and function are increased with exercise.[11] It could be cautiously suggested that platelet activation may indeed play an important role in the risk of cardiac events during strenuous exercise. Interestingly, aspirin, the most commonly prescribed anti-platelet medication has been shown to be ineffective in blunting platelet activation in response to exercise and this occurred in both normal individuals and cardiac patients.[116]

The mechanism of hyper-activation of platelet aggregation with exercise in cardiac patients is not fully understood, but might be linked to pro-aggregatory effects on platelets at the site of arterial stenoses. It has also been suggested that dysfunctional endothelium with a resultant insufficient release of anti-platelet compounds may be involved in the activation of platelets in cardiac patients. Platelet hyper-activation in cardiac patients was demonstrated by an increase in platelet factor IV in cardiac patients, but not in normal controls in response to exercise and recovery. On subgroup analysis, the increase in platelet factor IV after exercise only occurred in patients who exhibited exercise-induced ischaemia. Therefore, it was concluded that the increase of platelet activation associated with exercise in cardiac patients is ischaemia-related rather than coronary artery disease per se. It should be noted, however, that this explanation cannot be reconciled with indirect evidence of previous studies.[116]

Platelet activation has been documented in ath erosclerosis and in patients with peripheral arterial disease. This has been shown by the increase in urinary excretion of platelet-derived thromboxane A2 metabolites, by the presence of platelet activation in vitro and by the increased expression of adhesion molecules on the surface of platelets.[117] Chronic heart failure is associated with an increased risk of thrombosis and thromboembolic events that may be related to the hyper-coagulable state. Baseline von-Willebrand and soluble P-selectin were
significantly elevated in patients with chronic heart failure when compared with controls. Exercise in these patients was associated with an increase in plasma fibrinogen, plasma viscosity and haematocrit, and this increase persisted 20 minutes into recovery. In addition, there was a positive correlation between exercise workload and the maximal changes in plasma viscosity in this group of patients. Thus, it was concluded that strenuous exercise should be avoided in view of the potential prothrombotic effects in this high risk group of patients. Although the resting level of platelet aggregation was higher in patients with syndrome X compared with coronary heart disease patients and normal controls, their platelet aggregation was decreased following exercise, a response that differed significantly from that of patients with coronary heart disease and normal healthy individuals. Although the mechanism(s) of increased resting platelet aggregation in patients with syndrome X is not completely understood, several abnormalities involving endothelial cell function, cardiac adrenergic activity and oxidative stress have been implicated. The reduction of platelet aggregation with exercise in patients with syndrome X was suggested as a protective mechanism to combat stress-induced acute coronary episodes.

Sheer-induced platelet aggregation was determined in 27 patients with obstructive coronary artery disease before and after light exercise (less than or equal to stage III of the modified Bruce protocol). Ex vivo platelet aggregation was determined in flowing blood as the time to occlude a collagen- and ADP-coated ring. In this method, the shorter the occlusion time the greater the aggregation of platelets. Although exercise workload was sufficient to induce myocardial ischaemia in only 14 patients, a reduction in platelet aggregation time at peak exercise was observed in all patients, but not in controls. Aggregation times did not correlate with haematocrit, or white blood cells and platelet count. It was concluded that light intensity exercise in these patients was sufficient to transiently evoke hyper-aggregation, which was independent of myocardial ischaemia. Haemodynamic factors interacting with coronary obstruction or dysfunction of the endothelial were suggested as possible aetiological factors responsible for this hyper-aggregation.

Several hypotheses have been suggested to explain the mechanism by which exercise may alter platelet responsiveness. For example, the possible link between platelet responsiveness and alterations in oxidant-antioxidant status was evaluated in physically inactive individuals following moderate and strenuous exercise protocols. A single bout of strenuous exercise evoked a significant increase of ADP and collagen-induced platelet aggregation. Interestingly, moderate exercise decreased platelet sensitivity to aggregation only with low concentration of the aggregating agents. These results concur with those previously reported in which platelet aggregation is usually enhanced with strenuous exercise, but diminished by moderate exercise. The enhanced platelet aggregation to strenuous exercise was attributed to the attenuation of platelet sensitivity to endothelial-derived nitric oxide, due to the lesser rate of availability. This explanation seems unlikely in light of the recent evidence that clearly demonstrated a rise in plasma nitric oxide with strenuous exercise. It was suggested that the modifications of platelet responsiveness after exhaustive exercise may be mediated by oxidatively modified low-density lipoprotein (LDL).

An increase in soluble P-selectin during exercise is usually considered as an objective marker of in vivo platelet activation. Exercise-associated hypoxia was proposed as a mechanism of the increase in P-selectin due to platelet activation. Despite aspirin administration, an intense bout of acute exercise induced a significant increase in platelet aggregation in patients with previous myocardial infarction, and this was attributed to platelet activation as a consequence of catecholamine release. The catecholamines probably do not increase platelet aggregation per se, but their effects might be secondarily through activation of platelet aggregating agents such as ADP and thrombin. Such a mechanism would help to explain why platelet aggregates are still formed during exercise despite aspirin administration. It was concluded that aspirin might have
limited antiplatelet and antithrombotic effects during exercise and similar situations associated with enhanced catecholamine release.\textsuperscript{[109]}  

Strenuous exercise enhances oxygen consumption, resulting in the generation of free radicals and oxidative stress, which are linked to membrane damage, lipid peroxidation and platelet hyper-aggregation. LDL is reported to activate platelet functions as reflected by an increase in thromboxane release. It appears that LDL possesses platelet-activating properties in disease-free individuals. Recently, it was shown that the platelet-activating factor of LDL resides more in the modified forms of LDL than in native LDL.\textsuperscript{[124]} Platelet aggregation by collagen was modestly enhanced and inhibition of platelet aggregation by SIN-1 (nitric oxide donor) was significantly decreased following a treadmill test. It was suggested that the attenuated response of platelets to nitric oxide during exercise might result in thrombotic complications in sedentary patients particularly those with endothelial cell dysfunction.\textsuperscript{[121]}  

Although heavy physical exercise activates platelets in patients with stable angina and healthy controls, aspirin was ineffective at inhibiting exercise-induced platelet hyper-aggregation. In addition, exposing the patient and controls to mental stress was associated with considerable inter-individual variability in platelet aggregation and function, but the response was more pronounced in angina patients.\textsuperscript{[109]} Available evidence also suggests that platelet susceptibility to aggregating agents in whole blood is enhanced with advancing age and this has been attributed to modifications of intra-platelet calcium homeostasis and platelet membrane fluidity.\textsuperscript{[125]} In contrast to the general opinion, patients with angiographically documented myocardial ischaemia and stable angina experienced a reduced platelet activity and enhanced sensitivity towards epoprostanol (prostacyclin) and nitric oxide, probably due to an increased release of endogenous platelet inhibitor mediators as a counter-regulation to platelet activation.\textsuperscript{[126]}  

There is increasing evidence that chronic atrial fibrillation is associated with a prothrombotic state. However, a treadmill test in patients with chronic atrial fibrillation was associated with no change in P-selectin.\textsuperscript{[127]} This result contrasts with the finding of earlier reports,\textsuperscript{[128]} which showed that exercise in chronic atrial fibrillation patients induced a significant increase in platelet sensitivity to aggregation with ADP and this coincided with a rise in B-TG. These discrepant results may be attributed to differences in age range of the study participants and also to the exercise protocol employed in the two studies. It is also noted that the inclusion and exclusion criteria for patients with chronic atrial fibrillation were different between the two studies.  

4.3 Exercise Training and Platelets  

In contrast to reports on the acute effects of exercise, only meagre data are available on the influence of regular exercise training and platelet aggregation and function. The beneficial effects of regular endurance exercise are mediated by several factors including physiological improvements in the advocated haemostatic equilibrium, possibly due to attenuation of thrombogenic factors and enhancing blood fibrinolytic potential. It was also reported that physically inactive individuals have about a 50-fold increase in the risk of sudden death and a 100-fold increase in the risk of acute myocardial infarction when they perform vigorous exercise.\textsuperscript{[129]} About 70% of the cases related to exercise-induced sudden death and myocardial infarctions can be attributed to the occlusion of the coronary arteries by platelet-rich thrombi.\textsuperscript{[129]}  

Two controlled randomised trials were designed to determine the effects of training on platelets.\textsuperscript{[130,131]} The results of these studies have clearly shown that physical training of moderate intensity in young healthy male and female individuals reduces platelet adhesion and aggregation at rest and in response to acute intense exercise. Interestingly, platelet adhesiveness and aggregation in men were enhanced by acute strenuous exercise prior to training, but this response was attenuated after training. Detraining was associated with reversed effects on resting and post-exercise effects.\textsuperscript{[130]} Data from the same laboratory indicated that platelet adhesiveness
on fibrinogen-coated surface and ADP-induced platelet aggregation in women during the mid-follicular phase were desensitised by physical training. In addition, acute exercise-induced platelet hyper-activity prior to training was no longer apparent post-training and these favourable alterations in platelet adhesiveness and function were reversed with de-training.\textsuperscript{[131]}

It is known that the study of platelet aggregation and activation is complicated, as platelets are affected by a multitude of physical and chemical factors, including sample collection, handling and processing. Furthermore, pre-analytical problems, such as artificial activation \textit{in vitro}, limit the value of recent studies concerned with exercise and platelet aggregation and functions. Available studies were designed to overcome some of these methodological problems and indicated that strenuous exercise activates platelets in sedentary, but not in trained active and healthy individuals.\textsuperscript{[100]} This effect may be attributed to a higher catecholamine response to exercise in physically inactive individuals compared with those who are physically trained. An earlier study in overweight, middle-aged men with mild hypertension also showed that low- to moderate-intensity exercise training was associated with diminished platelet aggregation.\textsuperscript{[132]} Physical training is also followed by increased epoprostenol, a potent inhibitor of platelet aggregation and also a rise of nitric oxide, which is also known to possess a potent antiplatelet effect.\textsuperscript{[130,131]} These results concur with earlier reports\textsuperscript{[133]} that demonstrated a significant decrease in platelet aggregation in response to an acute bout of exercise following endurance training for 12 weeks. Likewise, the associated unfavourable adverse effects of ageing on platelets appear to be attenuated with physical training.\textsuperscript{[134]}

Regular exercise and physical conditioning may reduce the risk of vascular thrombotic events and protect against cardiovascular disease. Recent evidence suggests that 10 weeks of exercise training in rats effectively increased plasma- and platelet-derived nitric oxide metabolite levels. Furthermore, exercise training may decrease oxidised LDL and consequently decrease platelet adhesiveness on fibrinogen-coated surface and ADP-induced platelet aggregation. These changes are likely to be mediated by enhancing nitric oxide bioavailability in platelets and improving the potential of platelet-derived nitric oxide release after exercise training.\textsuperscript{[135]} Exercise training can invoke beneficial effects on platelets via different mechanisms. For example, it is known that exercise performed on a regular basis increases high-density lipoprotein cholesterol, which can stimulate epoprostenol production and thereby decrease platelet aggregation. In addition, exercise increases the release of nitric oxide, a potent mediator of anti-platelet effects and suppression of platelet reactivity.\textsuperscript{[130,131]} However, the resting and exercise-induced reductions in platelet aggregation reverse back to the pre-training level after de-conditioning.\textsuperscript{[136]}

5. Conclusion and Recommendations for Future Research

Blood haemostasis and its regulation are complex, with different pathways and mechanisms involved, each of which may be potentially controlled by various regulatory factors. Disturbance of blood haemostasis is recognised not only as a coronary risk factor, but also as a risk predictor of cardiovascular disease. Studies examining the acute effects of exercise on blood haemostasis have demonstrated changes in blood coagulation, modifications in blood fibrinolysis and alternations in platelet aggregation and functions. Among the reasons why blood haemostasis may be of particular importance in exercise physiology is that physical training is alleged to be associated with favourable modifications in blood clotting, fibrinolysis and platelets. However, experimental evidence available regarding the effects of physical training on blood haemostasis is incomplete and mostly fragmented. Even if relationships among markers of blood haemostasis and training were suggested in some earlier studies, the causality of the observed associations may not always be easy to explain. Furthermore, the mechanisms via which training-associated changes in blood haemostasis occurs remain to be elucidated, and the results reported should only be considered as pre-
liminary research findings. This is undoubtedly due to methodological variations including differences in training programmes, populations studied and the analytical methods employed for the determination of haemostatic indices. The hypothesis regarding the favourable effects of training on blood haemostasis should be further examined and available studies should be replicated.

Several questions related to exercise and platelets, particularly platelet aggregation and functions, remain unanswered and warrant further investigations. Platelets hyper-aggregation and hyper-activation have been identified as a cardiovascular risk in many studies. Based on research conducted to date, it is reasonable to conclude that acute exercise is associated with hyper-activation and hyper-aggregation particularly in patients with cardiovascular complications. In light of the meagre data reported, no valid conclusion can be drawn on the effects of physical training on platelet aggregation and function. An essential requisite in future studies should be controlled randomised trials with appropriate sample size. Furthermore, the possible preventative and therapeutic utility of exercise training warrants further investigations using standardised measurements. There are many exciting areas for future endeavours in this field. As noted above, an expansion of our knowledge about the effect of exercise training on platelet behaviour in health and disease is required. Among other things, we need a better understanding of the mechanism(s) responsible for platelet response to exercise and training. Future research should also be designed to determine the combined effects of exercise training and dietary manipulation on blood haemostasis in health and disease.

Specifically, the following questions should be answered in future research:

1. What kind of disease in relation to what kind of exercise leads to a clear increase in thrombin generation and thrombin potential?

2. What are the exact mechanisms underpinning hyper-fibrinolysis following exercise, and when and how is it suppressed?

3. What are the mechanisms for the increase in platelet reactivity and what are the meaning and the mechanisms of conjugate formation and dissolution?

The knowledge about the changes in blood haemostasis in response to different modalities of physical training will definitely be essential in future research.

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