Aging, physical conditioning, and exercise-induced changes in hemostatic factors and reaction products

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Van den Burg, P. J. M., J. E. H. Hospers, W. L. Mosterd, B. N. Bouma, and I. A. Huisveld. Aging, physical conditioning, and exercise-induced changes in hemostatic factors and reaction products. J Appl Physiol 88: 1558–1564, 2000.—The influence of age on training-induced changes in resting and stimulated hemostatic potential was studied in three age categories (Cat I–III; 20–30 yr, 35–45 yr, and 50–60 yr, respectively) of sedentary men before and after 12 wk of training. Coagulation, fibrinolytic activity, and activation markers (reflecting fibrin formation and degradation) were determined. Physical conditioning resulted in a more pronounced increase in von Willebrand factor (vWF) and factor VIII clotting activity (FVIII:c) in Cat I and II and a more pronounced shortening of the activated partial thromboplastin time in all categories at maximal exertion and during recovery. Enhanced increases in tissue-type plasminogen activator (t-PA) antigen and activity and single-chain (sc) urokinase-type plasminogen activator (u-PA) at maximal exercise and 5 min of recovery were observed in all age groups after training. The effects on FVIII:c, vWF, and scu-PA were most pronounced in the youngest age group (Cat I). Increases in the marker of thrombin generation were highest in Cat III; no effect was seen on thrombin-antithrombin complex, plasmin-antiplasmin complex, and D-dimer in any of the age groups. We concluded that training enhances both coagulation and fibrinolytic potential during strenuous exercise. The effect on FVIII/vWF and t-PA/u-PA is most pronounced in younger individuals, whereas thrombin formation is most pronounced in older individuals.

activation markers; coagulation; fibrinolysis

THE EFFECT OF PHYSICAL CONDITIONING on various cardiovascular risk factors has been studied extensively. Beneficial effects of training have been observed on lipid profile, hypertension, and insulin resistance, factors that are linked to cardiovascular complications (11, 19, 21, 32, 33). As a result, large numbers of sedentary individuals are, irrespective of age, advised to participate in training activities.

More recently, physical inactivity has been declared an independent risk factor in the pathogenesis of cardiovascular diseases, suggesting that additional unknown risk factors are involved (5). A disturbance in the hemostatic balance associated with increased fibrin deposition and enhanced thrombogenesis might constitute such an important risk factor (3, 8). Aging is also associated with adverse changes in coagulation and fibrinolysis that are suggestive of a thrombophilic state (1, 10, 26, 30). We therefore studied the effects of regular physical exercise on the balance between two systems that play a crucial role in thrombogenic processes, i.e., coagulation and fibrinolysis, in relation to age. A decrease in thrombogenic potential would further support the concept that “regular exercise is good for heart and blood vessels.”

Activation of coagulation results in the formation of thrombin, an enzyme that converts fibrinogen into (insoluble) fibrin. Activation of this system is reflected in enhanced levels of active clotting factors and of activation markers such as prothrombin activation fragment 1+2 (F1+2), which is a marker of thrombin generation, and thrombin-antithrombin complex (TAT), associated with a decrease in clotting time. When the fibrinolytic system is activated, plasmin, which is responsible for the degradation of fibrin, is formed. Activation of this mechanism is reflected in increased levels of tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) and activation products such as plasmin-α2-antiplasmin complex (PAP) and D-dimer (a cross-linked fibrin degradation product). Results of predominantly cross-sectional studies are suggestive of a beneficial antithrombotic effect of physical training on fibrinolytic components such as t-PA and/or plasminogen activator inhibitor (PAI-1), at rest and during acute exercise (6, 10, 14, 20, 26–28). Whether this is indeed associated with enhanced fibrin degradation remains to be determined.

Less attention has been paid to the effect of physical conditioning on coagulation activity, and the results are scarce and far less consistent (6, 10, 14, 27).

In this study, we determined plasma levels of hemostatic variables that are known to change during acute exercise, i.e., factor VIII clotting activity (FVIII:c), von Willebrand factor (vWF), activated partial thromboplastin time (APTT), t-PA, and u-PA (6, 27, 30). Recently, sophisticated tests for the determination of activation markers and activator-inhibitor complexes that reflect in vivo activation of coagulation and fibrinolysis have become available. The influence of acute exercise and/or training on the actual in vivo thrombin formation and fibrin degradation has been determined separately and...
simultaneously, but the results are conflicting and not consistent (9, 13, 16, 18, 20, 23).

An important aspect that has to be taken into consideration when training studies are performed is the fact that aging is associated with a thrombophilic state that may contribute to cardiovascular complications (1, 10). Another aspect that may determine the outcome of training studies and thus requires careful attention is changes in body composition related to weight loss and changes in dietary habits (15, 30, 33). The purpose of our study was to investigate the influence of aging on training-induced adaptations in the hemostatic system, both at rest and under stimulated conditions. We designed a highly standardized testing and training program and included three well-defined age categories of sedentary men in our study.

Coagulation and fibrinolytic factors, which are activated during exercise, as well as activation markers, were determined at rest, during acute submaximal and maximal exercise, and during the subsequent recovery period, before and after a 12-wk of training period.

**METHODS**

Participants

Sedentary men in three age categories [20–30 yr (Cat I), 35–45 (Cat II), and 50–60 yr (Cat III)] were recruited by an advertisement in a local newspaper. Respondents were asked to fill in a questionnaire providing information about their daily activity pattern and other health and lifestyle factors. Subjects were characterized as sedentary if they worked in sedentary jobs and did not participate in any form of sporting activity during leisure time. Other inclusion criteria were: no sporting activities during the previous 5 yr, apparently healthy, no medication, nonsmoking, and only moderate alcohol use. The study was approved by the Ethical Commission of the Utrecht University Hospital and participants joined the study after written informed consent was obtained.

Training and Test Procedure

Training. All subjects participated for 12 wk in the supervised training sessions performed in the laboratory. Participants exercised twice a week for 1 h at a constant submaximal level. During each training session, the work rate was adjusted for each individual to maintain a heart rate corresponding with that at 60–70% maximal oxygen uptake ($V_{O2\text{max}}$). At this submaximal level, on the basis of advice for recreational sporting activities, dear training-induced changes can be expected (2). Anthropometric measurements, $V_{O2\text{max}}$ test, and exercise test (Ex-test) were performed before the test, and exercise test (Ex-test) were performed before the test, and after a 12-wk of training period.

Anthropometric measurements. Height, body mass, and four skinfolds were measured as described previously (4), and body mass index (kg/m$^2$) and fat percentage were calculated.

$V_{O2\text{max}}$ test. $V_{O2\text{max}}$ was determined with an increasing work load test on a cycle ergometer (Lode, Groningen, The Netherlands). Subjects started with an initial load of 1 W/kg (60 rpm), which was increased every 2 min with 1 W/kg. After an age-dependent threshold was reached, the load was increased 2 W/kg every 2 min until participants reached their maximal performance. This threshold was 150 beats/min for Cat I, 135 beats/min for Cat II, and 110 beats/min for Cat III. Maximal exercise was indicated by a respiratory exchange ratio level >1.15, age-predicted maximal heart rate (HR$_{\text{max}}$), or when participants were unable to continue. Participants were encouraged to exert themselves maximally. The total work capacity (TWC) was calculated, at each step during $V_{O2\text{max}}$ test, as the product of load (W = 1/s) and time (s). The total amount of work (in J) is expressed per kilogram body mass. Ventilatory parameters were determined with Oxycon-$\beta$ (Mijnhardt, the Netherlands), which was calibrated before and after each test. Electrocardiogram was monitored continuously by using three leads (CCS, CMS, and CBS) with a megacart electrocardiograph (Siemens, The Netherlands).

Ex-test. This standardized test, designed for the collection of blood samples at rest, during submaximal exercise, at maximal exercise, and during recovery, was scheduled between 8:00 and 10:00 AM. Participants refrained from alcohol and coffee for 12 h before the test. On the day of the test, they had a light breakfast consisting of tea and toast. Ex-tests were performed on a cycle ergometer and comprised four consecutive periods: 1) the initial 10 min, during which the load was gradually increased until the HR was reached that corresponded with the participant’s HR at 70% $V_{O2\text{max}}$; 2) 15 min of exercise, during which the load was continuously adjusted to maintain a constant HR corresponding with 70% $V_{O2\text{max}}$, representing submaximal exercise intensity; 3) a period during which the load was increased in four steps of 1 min to attain, the HR corresponding with the HR at each participant’s $V_{O2\text{max}}$, representing maximal exertion; and 4) the recovery period, which comprised 10 min of active recovery whereby participants cycled at a load of 1 W/kg followed by 15 min passive recovery during which the participants remained upright.

During the Ex-test, blood was drawn immediately before the test, at 25 min, immediately after maximal performance, and at 5, 15, and 25 min of recovery.

Blood collection procedure. Blood samples were drawn (without stasis) by clean venipuncture (B-D Microlance 19 gauge) from the antecubital vein. The first 3 ml of each blood sample were discarded. Blood was collected in tubes containing chilled 3.8% (0.11 mol/l) trisodium citrate and in EDTA-coated tubes. For the determination of t-PA activity (Act), 1 ml of citrate blood was immediately mixed with an equal amount of sodium acetate buffer (0.2 mol/l, pH 3.9). Blood for PAI-1 antigen (Ag) determinations was collected in tubes containing citric acid, theophylline, adenosine, and dipyridamole (Becton-Dickinson). Within 10 min after collection, plasma was separated from centrifugation at 2,000 g for 20 min at 4°C, divided in small aliquots of 200 µl, and snap frozen in liquid nitrogen to be stored at −80°C.

Hematologic parameters. Samples from each individual obtained before and after 12 wk of training were tested simultaneously in one run to eliminate the intra-assay variation.

Coagulation activities of FVIIIc and vWF as well as APTT were determined, according to the manufacturer’s instructions, with a laser-nephelometric centrifugal ACL 200 analyzer (Instrumentation Laboratory, IJsselstein, The Netherlands). Deficient plasmas were obtained from Organon Teknika Nederland (Boxtel). Cephalin, calcium chloride, and calcium thromboplastin were provided by Instrumentation Laboratory. Blood for the normal plasma pool was donated by 40 healthy men. F$_{1+2}$ was determined with Enzymnost F$_{1+2}$ (Behring). TAT was determined with Enzymnost TAT micro (Behring).
t-PA Ag was measured with a commercial ELISA (Imulyse t-PA, Biopool, Umeå, Sweden), and t-PA Act was determined by using a commercially available kit (Cosset t-PA, Chromogenix). PAI-1 Ag was determined with CoaAELISA (Behring), and D-dimer was determined with Innogenetics (Innotest D-dimer, Biopool). PAP was determined with EIA APP micro-ELISA (Biopool). u-PA Ag and single-chain u-PA (scu-PA) Ag were determined with TintElize uPA and Chromolize uPA, respectively (Biopool). APTT was determined with TintElize uPA and Chromolize uPA, respectively (Biopool). u-PA Ag and single-chain u-PA (scu-PA) Ag were determined with TintElize uPA and Chromolize uPA, respectively (Biopool). PAI-1 Ag was determined with CoaAELISA (Behring), and D-dimer was determined with Innogenetics (Innotest D-dimer, Biopool). Hb and hematocrit were determined with a Sysmex NE 8000 (Toa Medical Electronics) analyzer. Changes in plasma volume were calculated according to Dill and Costill (12).

Statistics

Statistical analyses were performed with Superior Performing Software Systems (SPSS) version 5.01. Deviations from normality of distribution were checked for each variable. The distributions of hematologic parameters, except for vWF, were slightly skewed, and for these variables log transformations were performed. Multiple ANOVA repeated measures were used for analysis of each variable. Differences in time (training) and between categories were calculated. Results are expressed as means ± SE. Two-sided probability values were considered significant at \( P < 0.05 \).

RESULTS

Participants

Four participants withdrew before the study started, and two quit the study because of lack of motivation. Ultimately, 39 participants (Cat I: \( n = 13 \); Cat II: \( n = 13 \); Cat III: \( n = 13 \)) completed the whole test and training program and were included in this study. No medical complications were observed during the 12-wk test and training program.

Anthropometric Measurements

Age-related differences were observed in percent body fat and lean body mass but not in total body mass. No change in any of the anthropometric parameters was seen during the training period. The age-related differences observed before training persisted after training (see Table 1).

Exercise Parameters

\( V_{O2\max}, HR_{max} \), and TWC were related to age, being significantly lower in the older age groups. Training resulted in a significant increase in both \( V_{O2\max} \) and TWC in all three age categories (see Table 1).

Hemostatic Variables and Reaction Products

Age-related effects on hemostatic variables and reaction products before the start of the training. Preexercise plasma level of PAI-1 Ag (Cat I–III: \( 43 ± 8, 61 ± 8 \), and \( 62 ± 11 \) IU/ml, respectively) and t-PA Ag were higher in Cat II and III, whereas levels of \( F_1 + 2 \) were higher in Cat III only (Figs. 1, 2, and 3).

Acute (sub)maximal exercise induced an increase in all variables under study with a simultaneous shortening of the clotting time (APTT).

Age-related effects on coagulation parameters after training. No effect of training was observed on basal (resting) or submaximal exercise (70% \( V_{O2\max} \)) plasma levels (see Fig. 1). At maximal exercise intensity and during the entire recovery period, a more pronounced increase was observed in FVIII:c and vWF (Cat I and Cat II only), accompanied by a more pronounced shortening of the APTT (Cat I, Cat II, and Cat III), compared with pretraining results.

Age-related effects on fibrinolytic parameters after training. No changes in basal (resting) or submaximal exercise (70% \( V_{O2\max} \)) plasma levels were observed after training (see Fig. 2). At maximal exercise intensity and during the initial 5 min of recovery, more pronounced increases in t-PA Ag, t-PA Act, and scu-PA Ag were seen after training. The magnitude of this training-induced increase in both u-PA Ag and scu-PA Ag was significantly larger in Cat I.

Age-related effects on reaction products after training. \( F_1 + 2 \) demonstrated an increase after 25 min of submaximal exercise in contrast to pretraining results and a more pronounced increase at maximal performance and part of the recovery period in the older age groups (Cat II and Cat III). Although TAT, PAP, and D-dimer levels tended to increase more at 12 wk of training, results did not reach significance level (see Fig. 3).

Plasma Volume

A decrease in plasma volume was observed that was maximal during maximal exercise, independent of age (see Table 1). This decrease was significantly more pronounced after training (10.7 ± 0.6 vs. 13.2 ± 0.6%)

Table 1. Anthropometric and exercise data

<table>
<thead>
<tr>
<th></th>
<th>Cat I (n = 13)</th>
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<th>Cat II (n = 13)</th>
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<th>Cat III (n = 13)</th>
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<tbody>
<tr>
<td></td>
<td>B</td>
<td>T12</td>
<td>B</td>
<td>T12</td>
<td>B</td>
<td>T12</td>
</tr>
<tr>
<td>Age, yr</td>
<td>26 ± 1</td>
<td>26 ± 1</td>
<td>40 ± 1</td>
<td>40 ± 1</td>
<td>54 ± 1</td>
<td>54 ± 1</td>
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<td>Body mass, kg</td>
<td>75.4 ± 2.2</td>
<td>75.3 ± 2.2</td>
<td>79.9 ± 1.7</td>
<td>79.0 ± 1.7</td>
<td>82.0 ± 2.9</td>
<td>81.0 ± 3.2</td>
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<td>LBM, kg</td>
<td>59.4 ± 1.3</td>
<td>60.4 ± 1.4</td>
<td>55.8 ± 1.5</td>
<td>56.7 ± 1.4</td>
<td>53.3 ± 1.4</td>
<td>54.1 ± 1.3</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>21.0 ± 1.2</td>
<td>19.5 ± 1.2</td>
<td>30.1 ± 1.4</td>
<td>28.1 ± 1.3</td>
<td>34.7 ± 1.0</td>
<td>32.6 ± 1.6</td>
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<td>V02max, ml·min⁻¹·kg⁻¹</td>
<td>48.4 ± 1.6</td>
<td>56.5 ± 1.7†</td>
<td>41.2 ± 1.7</td>
<td>47.2 ± 1.9†</td>
<td>31.2 ± 1.1</td>
<td>36.6 ± 1.4†</td>
</tr>
<tr>
<td>TWC, Joule</td>
<td>122 ± 9</td>
<td>177 ± 10†</td>
<td>106 ± 9</td>
<td>139 ± 10†</td>
<td>73 ± 5</td>
<td>90 ± 7†</td>
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<td>HRmax, beats·min⁻¹</td>
<td>192 ± 2</td>
<td>195 ± 1</td>
<td>186 ± 2</td>
<td>189 ± 2</td>
<td>172 ± 3</td>
<td>176 ± 2</td>
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</table>
and of comparable magnitude for all three age categories. Correction applied for hemoconcentration did not significantly affect the outcome of the results.

**DISCUSSION**

The purpose of this study was to evaluate the influence of age on training-induced changes in resting and stimulated coagulation and fibrinolytic potential.

Our study comprised three well-defined age groups of 13 sedentary male subjects each, who were tested before and after 12 wk of submaximal (70% VO_{2max}) aerobic cycle ergometer training twice a week. Hemo-
static factors representing in vitro coagulation and fibrinolytic activity, and activation markers reflecting thrombin and plasmin generation as well as fibrin degradation in vivo, were determined under both rest-

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**Fig. 1.** Age-related effect of training on coagulation. FVIII:c, factor VIII clotting activity; NP, normal pool; vWF, von Willebrand factor activity; APTT, activated partial thromboplastin time; submax, submaximal exercise. I, 20–30 yr; II, 35–45 yr; III, 50–60 yr. *Difference between pretraining (solid lines) and posttraining (dashed lines), P < 0.05.

**Fig. 2.** Age-related effect of training on fibrinolysis. t-PA, tissue-type plasminogen activator; u-PA, urokinase-type plasminogen activator; scu-PA, single-chain u-PA; Ag, antigen; Act, activity. *Difference between pretraining (solid lines) and posttraining (dashed lines), P < 0.05.
Aging is associated with unfavorable changes in coagulation and fibrinolytic components that may play a role in the enhanced risk for thrombotic events (1, 10). In contrast, regular physical activity is generally accepted as part of a favorable lifestyle that is known to reduce the risk for cardiovascular diseases (5, 11, 21, 22, 33). Both a decrease in coagulation activity and an increase in fibrinolytic activity are associated with a reduced thrombogenic tendency. A number of authors have suggested that training-related adaptations, mainly in fibrinolytic potential, could be responsible for the reduced risk for coronary artery thrombosis (3, 7, 8, 33).

The majority of these studies have focused on the t-PA and its main inhibitor PAI-1 either at rest or under stimulated conditions (6, 20, 25–29). In contrast to fibrinolysis, the coagulation system has received little attention. The studies performed so far report no change or a slight increase in coagulation potential after training (6, 14, 20, 27, 29).

Although training-related adaptations in coagulation and fibrinolytic potential seem evident, the influence of aging (associated with an enhanced thrombogenicity) on these training-related processes has received little attention and has only been studied in a cross-sectional setting (10, 26).

It is also interesting to know whether the observed adaptations are indeed reflected in changes in the actual in vivo fibrin formation and/or degradation. Influence of Aging on Hemostatic Variables Before the Start of the Training Program

Before the start of the training, the older participants demonstrated a less favorable hemostatic profile reflected in significantly higher constitutive (resting) F1\textsubscript{2}, PAI-1, and t-PA levels, underscoring earlier observations (1, 10, 26).

During exercise, the well-known increases in the in vitro coagulation (vWF, FVIII:c, and APTT) and fibrinolytic (t-PA and u-PA) activation were observed, comparable for all age groups. Significant changes were also seen in the activation markers TAT, PAP, and D-dimer, but not in F1\textsubscript{2}, indicating that additional fibrin degradation in vivo did occur with only marginal or no prothrombin conversion, which support the findings in most studies (9, 13, 24) but is not supported by the results in other studies (16, 18, 23).

During recovery, the enhanced fibrinolytic activity returned to baseline levels (in contrast to the coagulation activity, which was still significantly enhanced at the end of the recovery) to a comparable extent in all three age groups. The results of exercise-induced changes in relation to age and the possible unfavorable effects of the unbalanced hemostasis have been presented and discussed previously (30).

Influence of Aging on Training-Induced Adaptations in Hemostasis

The significant increase in \(\text{VO}_{2\text{max}}\) in all three age categories clearly indicated that training had been
equally effective in improving cardiopulmonary fitness. It is important to note that no changes in either body mass or body fat percent were observed in any of the three groups, given that changes in body composition per se are closely associated with changes in coagulation and fibrinolysis (10, 15, 30).

Resting and submaximal exercise. With the exception of a significant increase in $F_{1-2}$ in the elderly, reflecting additional thrombin formation, no effect of training was observed on constitutive and submaximal plasma levels of coagulation variables in either group, which underscores previous results obtained in longitudinal studies (14, 29).

No effect was seen on resting and submaximal levels of fibrinolytic parameters, which is in agreement with some but at variance with others who reported enhanced fibrinolytic activity (6, 26–28). The majority of these studies are, however, cross-sectional with the exception of that of Stratton et al. (26), who performed a longitudinal training study and observed an increase in fibrinolytic activity in elderly only but not in young subjects. Unfortunately, few authors apply corrections for changes in body mass and composition observed after conditioning or for differences in anthropometry between trained and untrained individuals. In addition, differences in experimental setup may also be responsible for the discrepant results. As mentioned before, the study of hemostatic variables requires a meticulous standardization. Differences in blood sampling procedures, e.g., clean venipuncture vs. the use of a catheter which may cause additional activation (17); intensity (strenuous vs. submaximal), or duration and type (running vs. cycling) of training could affect the outcome of the studies.

Maximal exercise and recovery. The increase in clotting potential that was observed during maximal exercise varied with age. FVIII:c and vWF were significantly more pronounced in the younger age groups (Cat I and II) only, although the decrease in the clotting time (APTT) was comparable for all three age categories. An explanation for the more pronounced increase in vWF and FVIII:c in the younger men could be a greater susceptibility to endothelial triggers, e.g., higher shear stress or an enhanced capacity of the endothelium to release vWF. The $F_{1-2}$ increase during maximal exercise and part of the recovery was highest in the oldest age category (Cat III). That the older men demonstrated a more pronounced increase in prothrombin activation is compatible with diminished levels of coagulation inhibitors as reported before (30).

The training-induced effect on scu-PA was also more pronounced in the youngest participants (Cat I). In contrast, (the smaller) training-related changes in t-PA were not related to age. This finding underscores the notion that the mechanisms responsible for the increase in t-PA and scu-PA during exercise are different, scu-PA levels being closer related with workload than t-PA (30, 31).

From the results of the present study we conclude 1) that aging is associated with unfavorable changes in constitutive plasma levels of hemostatic variables; 2) that the acute exercise-induced changes are superimposed on the resting levels; 3) that after 12 wk of submaximal aerobic training, no changes are observed in constitutive (resting) plasma levels of the hemostatic variables and activation products under study in either young or elderly healthy individuals; 4) that both the hypercoagulability and hyperfibrinolysis induced by (acute) exercise are enhanced after 12 wk of submaximal training and maintained at a higher level during recovery; 5) that the exercise-induced increase in hemostatic activators after training is more prominent in younger individuals, suggesting a greater plasticity and susceptibility to exercise stimuli; 6) that thrombin formation is accelerated in trained individuals in relation with age, whereas fibrin(ogen) degradation is not affected; 7) that aging seems to be associated with additional risks during maximal exertion because lower increases in FVIII:c and vWF are needed for comparable shortening of the clotting times and the fibrinolytic response of scu-PA is less pronounced; and 8) that the disparate decrease of coagulation and fibrinolysis during the recovery period seems aggravated in trained individuals.

From the results, we cannot infer a direct beneficial or detrimental effect of recreational physical conditioning in healthy subjects. It would, however, be advisable for older individuals with an enhanced thrombotic tendency and at risk for cardiovascular complication to avoid extreme exertional activity.

Regular submaximal exercise remains nevertheless advisable because hemostatic changes are relatively small and clear beneficial effects on hypercholesterolemia, hypertension, insulin resistance, and body composition have been reported (11, 19).

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