Fibrinolytic and Coagulation Factors in Very Old Subjects: Association With Lipoprotein Profile and Anthropometric Variables

Luciana Vergnani, Giovanni Zuliani, Franco Ricci, Nadia Manzoli, Marcello Carantoni, Stefano Volpato, and Renato Fellin

Second Department of Internal Medicine, University of Ferrara, Italy.

**Background.** We evaluated plasminogen activator inhibitor-1 (PAI-1), factor VII activity (FVII), and fibrinogen in a sample of octo-nonagenarians. Furthermore, we investigated the relationship of these fibrinolytic and coagulation parameters with lipoprotein profile and anthropometric variables in the absence or presence of disability.

**Methods.** We enrolled a population of 162 octo-nonagenarians, divided in two groups on the basis of presence or absence of disability in the activity of daily living (ADL). All the anthropometric determinations were carried out according to standardized methods. Blood samples for hemostatic and lipid determinations were collected after overnight fasting and resting.

**Results.** PAI-1 activity and fibrinogen levels were significantly higher in disabled (DIS) compared to free-living (FL) adults, whereas FVII did not show differences in the two groups. PAI-1 activity and FVII positively correlated to anthropometric parameters (body mass index, subscapular and tricipital skinfold thickness) in both DIS and FL. No correlations were found between fibrinogen and other variables in FL, whereas a negative relation with high density lipoprotein-cholesterol levels emerged in DIS. FVII was positively related with total cholesterol low density lipoprotein-cholesterol, and apolipoprotein B in both FL and DIS.

**Conclusions.** In a sample of octo-nonagenarians, PAI-1 activity and FVII show a significant correlation with several anthropometric and lipoprotein parameters, suggesting that these variables are strongly associated with body composition and lipid metabolism independent from age and disability. DIS presented higher PAI-1 and fibrinogen levels; this observation may take in account the high prevalence of vascular diseases and also occult inflammation, which are known to affect these parameters.

Blood coagulation and fibrinolytic systems are involved in the process of atherosclerosis. Hypercoagulability and hypofibrinolysis play important roles in the progression of atherosclerosis and in the development of atherosclerotic disease such as coronary heart disease (CHD), stroke (1,2) and peripheral arterial disease (3). Coagulation and fibrinolysis abnormalities have been demonstrated in patients with hypercholesterolemia and hypertriglyceridemia; it has been suggested that an altered balance in fibrinolytic and coagulation systems, associated with alterations of lipid metabolism, increase the risk of cardiovascular disease (1,4).

Plasminogen activator inhibitor-1 (PAI-1) is the main regulator of fibrinolytic function: increased PAI-1 plasma activity is responsible for the impaired fibrinolytic activity associated with acute myocardial infarction (5), deep vein thrombosis (6), and diabetic complications (7). Furthermore, conditions such as obesity, glucose intolerance, and acute ischemic stroke are associated with a rise in PAI-1 levels (8,9). PAI-1 circulates following circadian variations during 24 h; a peak of PAI-1 has been detected before acute myocardial infarction (10) suggesting that this protein is directly involved in the thrombogenetic process rather than acting as an acute phase substance.

Since the Northwick Park Heart Study demonstrated that high plasma level of fibrinogen is independently related to vascular diseases, this substance has acquired a direct role in atherogenesis (11,12). Among the coagulation factors, factor VII coagulant activity (FVIIc) has been reported as a good predictor for coronary heart disease. Gender, glucose intolerance, obesity, and hyperlipemia are all conditions associated with elevated FVII levels and increased risk of death for ischemic heart diseases (11,12). Most studies on the hemostatic system and its relationship with the metabolic profile have been performed in middle-aged adults or in patients with CHD, obesity, hypertension, and diabetes (13-15). The aging process is known to influence coagulation and fibrinolytic parameters along with insulin plasma levels (16,17). Fibrinogen and FVII are considered to rise in elderly people (16,18). Nevertheless, much of the known data do not take in account the health status and the possible presence of disability.

The aim of the present study was to evaluate the hemostatic profile, including fibrinolytic factor (PAI-1), coagulation factor (FVIIc), and fibrinogen, in a sample of very old individuals aged over 80 years, and to investigate its relationship with lipoprotein and anthropometric variables in the absence or presence of disability.
METHODS

The Val Vibrata Aging Project is a longitudinal observational study initiated in 1992 with the aim of evaluating metabolic, anthropometric, and hemostatic parameters, as well as plasma lipid peroxides, in a sample of strictly selected free-living healthy octo-nonagenarians, and describes the impact of these factors on future disability, morbidity, and mortality. In the initial cross-sectional part of the study, the free-living (FL) were compared with a group of disabled (DIS) matched for age and origin. Results about plasma lipoprotein (a) levels and apolipoprotein (a) [apo(a)] isoforms, and plasma lipid peroxides and antioxidant systems, have been described elsewhere (19,20).

Val Vibrata is a valley located in the province of Teramo (Abruzzo region, central Italy). From a sample of about 15,000 residents referring to 10 family doctors (Associazione Medica Sabin), 162 octo-nonagenarians were enrolled during the years 1992-1993. One group included 100 FL (58 males and 42 females: average age 84.2 ± 2.7 and 85.5 ± 3.7 years, respectively). The inclusion criteria for FL were as follows: (a) age ≥ 80 years; (b) origin from the Vibrata valley or the Abruzzo region; (c) negative history for cardiovascular, severe respiratory and neurological diseases, dementia, neoplasm, diabetes mellitus, major depression, and alcoholism; (d) absence of specific changes in blood cells count, clinical chemistry parameters, urine analysis and neoplastic markers suggestive of any occult disease; and (e) absence of disability: only subjects in A-B class of the Katz index were enrolled (21).

The second group consisted of 62 DIS (24 males and 38 females: average age 86.6 ± 4.2 and 86.9 ± 5.0 years, respectively). The inclusion criteria for DIS were as follows: (a) age ≥ 80 years; (b) origin from the Vibrata valley or Abruzzo region; and (c) presence of severe disability, class F-G of the Katz index. In the DIS the most frequent pathologies were dementia (51%), severe degenerative arthritis (42%), CHD (28%), stroke and transient ischemic attacks (24%), chronic bronchitis (18%), and obstructive peripheral arteriopathy (8%).

After overnight fasting and resting, blood samples were collected for hemostatic and lipid determinations were collected without stasis from an antecubital vein between 8:00 and 9:00 a.m. into siliconized vacuum tubes containing 3.8% trisodium citrate (9 vol. blood to 1 vol. .13 M trisodium citrate) for the hemostatic factors and in regular tubes for lipids determinations. Blood was kept for 1 h at 4 °C and centrifuged at 3,000 × g for 15 min, and then plasma was stored at -80 °C until use. Determinations of lipoprotein parameters were carried out within 3 h, whereas the hemostatic factors were assayed within 1 year of blood collection.

At the time of the assay, samples were transferred to a water bath at 37 °C for 10 min. PAI-1 and FVII activities were assayed by a chromogen substrate assay using an automated device (Behring Chromo Time System, Behring Institute, Scoppito, Italy). The interassay coefficients were between 3% and 6% for PAI-1 and 4% for FVII.

Fibrinogen concentration has been determined by radial immunodiffusion (NOR-Partigen plates, Behring Institute, Scoppito, Italy). The interassay coefficient was 8%. Total cholesterol and triglycerides were measured on a Shimadzu analyzer (model CL7000; Tokyo, Japan) by an enzymatic procedure. High density lipoprotein (HDL)-cholesterol was evaluated after a selective lipoprotein precipitation with polyanions (sodium heparin and dextran sulphate). ApoA-I and apoB were assayed by rate-nephelometry (Kallestad analyzer, model QM300 Pasteur, Paris, France). Serum fasting insulin was measured by a radioimmunoassay using a commercially available kit (Techno Genetics, Recordati, Italy).

All the anthropometric determinations were carried out by the same two physicians over the entire study according to standardized methods (22). Body mass index (BMI) was calculated as weight (kg) divided by the square of the height (m). Subscapular skinfold thickness (SS) was determined from the average of triple measurements, picking just below the inferior angle of the scapula using an Harpenden calliper. Tricipital skinfold thickness (TS) was determined in the midline of the posterior aspect of the arm, over the triceps muscle, midway between the lateral projection of the acromion process of the scapula and the inferior margin of the olecranon process of the ulna. Waist circumference was calculated between the lower rib and the iliac crest at the end of a normal expiration. The hip circumference was evaluated as the largest measurement in a horizontal plane around the buttocks. The waist/hip ratio (WHR) was then used.

The values were expressed as mean ± standard deviation (SD). Triglycerides and PAI-1 activity values were log-transformed to approximate a normal distribution. The Mann-Whitney test was used to compare the mean values in the two selected groups. Simple linear regression was used to check the correlation between variables. Stepwise multiple regression analysis (with forward introduction) was used to check the contribution of lipoprotein and anthropometric parameters to the variability of PAI-1 in FL; SS, TS, BMI, apoB, and insulin were considered independent variables.

RESULTS

Figure 1 reports coagulation and fibrinolytic parameters in the two groups of octo-nonagenarians. The mean PAI-1 activity turned out to be in the normal variability range (0.3-3.5 U/mL) in both the study groups (1.99 ± 0.9 U/mL in FL and 2.7 ± 1 U/mL in DIS, respectively), but DIS showed a significantly increased PAI-1 activity compared to FL (p < .01). In the DIS, a 4-fold increase in the prevalence of subjects with PAI-1 activity exceeding the range of normal variability, compared to FL, was found (24% vs 6%, respectively). Even if mean fibrinogen plasma levels were in the regular range, DIS had significantly (p < .05) higher concentrations than FL (2.59 ± .49 g/L and 2.98 ± .90 g/L in FL and DIS, respectively). FVII coagulant activity was normal and did not describe any difference between the two groups.

We have previously reported significant differences in lipoprotein profile between the FL and the DIS octo-nonagenarians: the latter were characterized by lower total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), HDL-C, and apoB levels, but higher triglyceride (TG) concentrations (23). Moreover, no differences in anthropometrics emerged between the two groups, with the excep-
tion of WHR and SS that were both higher in the FL (Table 1). Fasting insulin plasma levels resulted of 6.17 ± 3.19 μU/mL in FL and 6.61 ± 3.37 μU/mL in DIS and did not show significant differences between the two groups studied.

To verify the relationships of lipoprotein, anthropometric variables, and insulin levels with the coagulation and fibrinolytic factors, we performed a simple linear regression analysis. Table 2 gives the main correlation between all the variables evaluated in our two groups. In the FL PAI-1 activity showed a positive correlation with SS (r = .28, p < .01), TS (r = .28, p < .01), BMI (r = .25, p < .05), apoB (r = .28, p < .01), and insulin (r = .36, p < .001). In the DIS PAI-1 activity still held the positive correlation to SS (r = .28, p < .05), TS (r = .31, p < .01), and BMI (r = .40, p < .001).

Stepwise multiple regression analysis was used to check the contribution of lipoprotein and anthropometric parameters to the variability of plasma PAI-1 levels in FL. Insulin and apoB were independently associated with PAI-1 levels, explaining 18% of total variability.

Fibrinogen did not show any relation with the lipoprotein or anthropometric parameters in FL. In the DIS fibrinogen concentrations were inversely related to HDL-C (r = -.31, p < .01), and positively correlated to WHR (r = .27, p < .05) and TS (r = .25, p < .05).

FVII activity was positively related with TC (r = .24, p < .05), LDL-C (r = .24, p < .05), and apoB (r = .26, p < .05) in the FL. Similar correlations were found in DIS, in which FVII not only was related to TC (r = .40, p < .001), LDL-C (r = .31, p < .01), and apoB (r = .44, p < .001), but also to TG (r = .36, p < .01) and to SS and TS (r = .26, p < .05).
DISCUSSION

In our free-living healthy octo-nonagenarians, PAI-1 activity was in the normal distribution range, suggesting that very old healthy people might have a good fibrinolytic activity; these results strongly support previous reports showing that PAI-1 does not increase with age (24). As expected, a significant correlation was found between PAI-1 activity and BMI in both FL and DIS; low fibrinolytic activity has been documented in obese people, showing that PAI-1 does not increase with age (24). As in very old healthy people, PAI-1 activity was in the normal range, suggesting that the association of skinfold thickness and BMI with PAI-1 activity is well understood; it has been shown that the synthesis of PAI-1 in obese people might be modified by a greater number of circulating lipoprotein particles.

Moreover, fasting insulin levels were positively related to PAI-1 activity in FL ($r = .36$; $p < .001$). These data also confirm in a population of very old healthy subjects the close relationship already described in previous cross-sectional studies on young or middle-aged humans (13). Insulin seems to stimulate the synthesis and the release of PAI-1, directly acting on endothelial cells and hepatocytes, or indirectly through its action on plasma triglycerides (8). Nevertheless, we found a significant correlation between insulin and TG, but not between TG and PAI-1; this observation seems to support the hypothesis of a direct effect of insulin on PAI-1 production. A direct correlation between TG levels and PAI-1 activity is still a matter of discussion: some authors emphasized a relationship between these two parameters in both middle-aged and elderly subjects (16, 25), whereas other publications reported the absence of such correlation (26).

In FL PAI-1 activity was significantly correlated ($p < .01$) with apoB plasma levels, whereas no significant PAI-1 changes were seen with respect to TC and LDL-C in both groups. This finding, to our knowledge, appears to be the first report of a positive association between apoB and PAI-1. There is good evidence indicating that modifications of the hemostatic system are related to the lipid profile: many authors have already documented the relationship between PAI-1 and TC levels (27). It is of interest in our observation that only apoB was related to PAI-1 activity in our sample. In fact, all the lipoproteins of hepatic origin detected in the plasma during fast, i.e., very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), and LDL, contain one molecule of apoB. This finding might suggest that PAI-1 activity can be related to the number of circulating particles and not to the amount of transported lipids, but more in vitro and in vivo valuations are needed to show a solid bilogical relation.

Stepwise multiple regression analysis revealed that insulin and apoB plasma levels in FL were independently associated with PAI-1 activity ($r^2 = .14$ and .21, respectively), whereas BMI, SS, and TS were not. These results suggest that the association of skinfold thickness and BMI with PAI-1 may be mediated by insulin. The statistically independent burden of apoB on PAI-1 activity is poorly understood; it has been shown that the synthesis of PAI-1 in endothelial cells and hepatocytes is enhanced by VLDL and LDL through their binding to the apoB/apoE receptor (28). We hypothesize that the apoB contribution to PAI-1 production is secondary to a stimulation of endothelium and liver by a greater number of circulating lipoprotein particles.

Even if the PAI-1 activity was in the normal range in both groups, DIS showed a significantly higher PAI-1; these data suggest that very old disabled subjects exhibit an impaired plasma fibrinolytic activity compared to healthy people matched for age. It is difficult to speculate in a cross-sectional study whether PAI-1 may be a marker for atherosclerotic diseases, or whether its rise is the consequence of a chronically stimulated endothelium.

It has been demonstrated that elevated levels of PAI-1, or its increased activity, are associated with the hypofibrinolysis observed in CHD, myocardial infarction, deep venous thrombosis, and stroke (5–7, 9). In our octo-nonagenarians, disability was largely associated with the presence of CHD.

| Table 1. Anthropometric Data and Serum Lipid and Lipoprotein Levels in Healthy FL and DIS Octo-Nonagenarians |
|---------------------------------|-----------------|-----------------|-----------------|
| **FL**                          | **DIS**         | **p-value**     |
| TC (mg/dL)                      | 219 ± 39        | 195 ± 44        | < .05           |
| LDL-C (mg/dL)                   | 143 ± 33        | 124 ± 38        | < .05           |
| HDL-C (mg/dL)                   | 53 ± 11         | 42 ± 7          | .05             |
| TG (mg/dL)                      | 111 ± 52        | 147 ± 70        | < .05           |
| ApoB (g/L)                      | .08 ± .27       | 1.02 ± .39      | —               |
| ApoA-1 (g/L)                    | 1.51 ± .30      | 1.34 ± .38      | < .05           |
| SS (mm)                         | 15.6 ± 6.7      | 13.2 ± 6.0      | < .05           |
| TS (mm)                         | 11.2 ± 5.1      | 11.4 ± 6.0      | —               |
| WHR                             | .96 ± .06       | .92 ± .08       | < .02           |
| BMI                             | 25.5 ± 3.8      | 24.4 ± 5.1      | —               |

Notes: Values are mean ± SD. TC, total cholesterol; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; TG, triglycerides; SS, subcutaneous skinfold; TS, tricipital skinfold, WHR, waist-hip ratio; BMI, body mass index.

| Table 2. Linear Regression Analysis Between PAI-1, FVII, and Fibrinogen, and Anthropometric and Metabolic Variables in FL and DIS Groups |
|---------------------------------|-----------------|-----------------|
| **FL**                          | **DIS**         | **p-value**     |
| PAI-1                           | FVII            | Fibrinogen      |
| CT                              | .06             | .24*            |
| ApoB                            | .28*            | .26*            |
| LDL                             | .02             | .24*            |
| HDL-C                           | .08             | .10             |
| ApoA-1                          | .10             | .07             |
| TG                              | .17             | .02             |
| SS                              | .28*            | .01             |
| TS                              | .28*            | .11             |
| BMI                             | .25*            | .06             |
| WHR                             | .03             | .06             |
| Insulin                         | .36*            | .03             |
|                                 | .05             | .14             |

Notes: Terms are defined in Table 1. Values are correlation coefficients ($r$; (-) is negative correlation. $*p < .05$; $t_p < .01$; $t_p < .001$.}
stroke, and obstructive peripheral arteriopathy, conditions in which PAI-1 activity had been demonstrated to increase (5,6,9). In DIS affected by CHD, the PAI-1 activity was higher than in those octo-nonagenarians suffering from all the other diseases, but the difference was not statistically significant (p = .07), suggesting that in our very old disabled CHD itself cannot justify the observed PAI-1 elevation. Moreover, the presence of occult inflammations may modulate the increased amount of circulating fibrin, thrombin, interleukin 1, bradykinin, free radicals, and cytokines; these substances are known to be able to stimulate the release of PAI-1 from endothelial cells (29,30).

In addition, the increased PAI-1 plasma levels in DIS might be secondary to the total lack of physical activity related to the presence of severe disability. It has been previously demonstrated that inactive subjects show higher PAI-1 activity compared to regularly active men (31); this finding supports the important effect provided by regular physical activity in modulating the fibrinolytic system. On the other hand, in DIS we did not find any correlation between insulin and PAI-1; furthermore, fasting insulin was not increased in DIS compared to FL even when PAI-1 activity was higher.

The presence of acute and chronic illnesses may modulate different endocrine responses that can lead to an increase of insulin counterregulatory factors, and may explain the lack of correlation between insulin and fibrinolytic system in DIS. Furthermore, in the DIS group apoB plasma levels still showed a relation with PAI-1 activity even if the correlation coefficient did not reach a significant value (r = .22). Considering that apoB and insulin levels were similar in both groups, we can conclude that these two parameters are not completely able to explain the rise in PAI-1 activity in the DIS group.

Mean fibrinogen levels were within the normal range in both groups; nevertheless, DIS showed significantly higher fibrinogen levels compared to FL. It is difficult to establish rigid criteria for the range of variability of fibrinogen because its concentration may be influenced by different laboratory methods (32). Moreover, fibrinogen has been indicated as an acute phase reactant, and fibrinogen concentrations may be increased by occult inflammatory state (32); the presence of clinically asymptomatic inflammations may have induced the rise of fibrinogen levels in the DIS group.

No correlations were found between fibrinogen and other variables in FL, whereas a negative relation with HDL-C levels emerged in DIS. Evidence suggests that the exposure to endotoxins causes a decrease in HDL-C levels (33); furthermore, it has been shown that elderly affected by chronic diseases often present occult inflammation and low plasma lipid levels (34). The inverse relationship between fibrinogen and HDL-C in DIS supports the hypothesis that low HDL-C levels and high fibrinogen may mark an acute phase response; lower albumin levels (data not shown) further support the presence of inflammation in DIS. Among the coagulant factors we measured, FVII activity was within the normal range in both groups and did not show any difference between FL and DIS.

FVII coagulant activity was positively related with CT, LDL-C, and apoB in both FL and DIS, suggesting that the relationship between this factor and the lipoprotein profile is not influenced by age or disability. These results confirm the findings of others who demonstrated the association of FVII with cholesterol and triglycerides (35), and the important role of larger apoB-containing lipoprotein particles such as VLDL or LDL on FVII levels. Together these observations support the idea that FVII may be activated by large negatively charged lipoprotein.

In conclusion, our data show that, in a sample of octo-nonagenarians, mean PAI-1, fibrinogen, and FVII are within the normal variability range and strongly reproduce their biological variability in healthy young adults. Furthermore, PAI-1 and FVII were significantly correlated with several anthropometric and lipoprotein parameters, suggesting that these variables are strongly associated with body composition and lipid metabolism independently from age and disability. Disabled octo-nonagenarians presented higher PAI-1 and fibrinogen levels; this observation may take in account that our sample was characterized by a high prevalence of vascular diseases and probably by occult inflammation, which are known to affect these parameters.

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Address correspondence to Prof. Renato Fellin, 2nd Department of Internal Medicine, University of Ferrara, Via Savonarola No. 9, 44100 Ferrara, Italy. E-mail: flr@ifeuniv.unife.it.

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Assistant Professor, College of Medicine
University of Nebraska Medical Center

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