

Hypercoagulability in Centenarians: The Paradox of Successful Aging

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With advancing age, an increasing number of healthy individuals have laboratory signs of heightened coagulation enzyme activity. Such biochemical hypercoagulability might be the basis of either the increased thrombotic tendency occurring with age or a harmless manifestation of this process. To see whether these alterations are also present in the very elderly who had aged successfully, 25 healthy centenarians were studied and results of coagulation and fibrinolysis measurements were compared with those obtained in two control groups of healthy adults, 25 ranging in age from 18 to 50 years and 25 from 51 to 69 years. Older controls had, in general, slightly higher values of several coagulation and fibrinolysis measurements than younger controls. Centenarians had striking signs of heightened coagulation enzyme activity, as assessed directly by measuring activated factor VII in plasma ($P < .01$, compared with either control

group) or indirectly by measuring the plasma levels of the activation peptides of prothrombin, factor IX, factor X, and thrombin-antithrombin complexes (all $P < .001$). Heightened coagulation enzyme activity was accompanied by signs of enhanced formation of fibrin (high fibrinopeptide A, $P < .001$) and secondary hyperfibrinolysis (high D-dimer and plasmin-antiplasmin complex, $P < .001$). Plasma concentrations of fibrinogen and factor VIII were higher than in controls, whereas other coagulation factors were not elevated. In conclusion, this study shows the very elderly do not escape the state of hypercoagulability associated with aging, but that this phenomenon is compatible with health and longevity. Hence, high plasma levels of the coagulation activation markers in older populations do not necessarily mirror a high risk of arterial or venous thrombosis.

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THE PROCESS OF aging in humans is accompanied by modifications of the blood coagulation system. It is well established that the plasma concentrations of some coagulation factors (such as factor VII, factor VIII, and fibrinogen) increase progressively with age.¹⁻³ However, these procoagulant proteins are normally present in plasma in such a large excess that the pathophysiologic significance of the relatively modest increases that occur with aging is uncertain. Using sensitive immunoassays for markers of coagulation enzyme activation, Bauer et al⁴⁻⁶ showed that with age advancing from 42 to 80 years, an increasing number of healthy men developed a laboratory picture of enhanced activity of coagulation enzymes, expressed indirectly by high plasma levels of the activation peptides that are cleaved from prothrombin, factor IX, and factor X when these zymogens are converted into the corresponding active enzymes.⁴⁻⁶

Because thrombotic disease increases with age, biochemical hypercoagulability in the elderly might be the basis for their increased thrombotic tendency. On the other hand, the phenomenon may simply be a harmless manifestation of the aging process. We attempted to evaluate these possibilities by studying coagulation and fibrinolysis in a group of healthy centenarians. Centenarians are an important natural model of successful aging and may help to identify crucial mechanisms for the maintenance of homeostasis in body systems.

For instance, the study of centenarians has helped to define the pathophysiologic relevance of autoimmunity during the aging process, as these individuals have much lower levels of organ-specific autoantibodies than younger individuals.⁷ We reasoned that finding laboratory signs of hypercoagulability in centenarians would favor the view that these signs are a manifestation of the natural aging process. On the other hand, absence of the abnormalities present in noncentenarian elderly individuals would indicate that there has been a selection process, leaving those individuals who aged without signs of hypercoagulability alive and healthy.

MATERIALS AND METHODS

Subjects. A total of 75 individuals were studied: 25 (the cases) were more than 100 years old, and 50 (the controls) were less than 100 years old. Approval for these studies was obtained from the Institutional Review Board of the University of Milan and informed consent was obtained according to the Declaration of Helsinki.

Centenarians. Centenarians (age range, 100 to 102 years, 9 men and 16 women) were ambulatory, self-sufficient, and lived in their homes. Followed at the Institute of Internal Medicine, they were chosen on the basis of the strict criteria set by the Senieur protocol.⁸ This protocol establishes admission criteria for gerontologic studies based on clinical history, physical examination, and laboratory data and sets limits for pharmacologic interferences. By setting strict criteria for choosing centenarians, our aim was to exclude endogenous and exogenous influences on the coagulation system and to standardize the population under study. Causes for exclusions were smoking, alcohol abuse, intake of drugs affecting hemostasis, and chronic disorders such as infection, inflammation, malignancy, cardiac insufficiency, dementia, diabetes, cataracts, renal, and liver disease. An electrocardiogram, hemocytometry, and urinalysis were obtained in all centenarians and erythrocyte sedimentation rate and plasma concentrations of glucose, sodium, potassium, albumin, alkaline phosphatase, alanine and aspartate aminotransferase, urea, and creatinine were measured. Centenarians with values outside the 2.5 and 97.5 percentile of the reference values were excluded.

Controls. Controls ($n = 50$, 20 men and 30 women) were blood donors or hospital staff members, and were chosen to make up two groups of equal size: younger controls, ages 18 to 50 years and older controls, ages 51 to 69 years. On the basis of history and physical examination, none of them had any of the conditions taken as causes of disqualification for the group of healthy centenarians. The same hematocchemical parameters were evaluated in the control population.

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Blood sampling and processing. Venous blood was obtained between 8:00 and 10:00 AM from the antecubital veins by two-syringe techniques using 19-gauge needles. Blood samples for factor IX and X peptide assays were drawn into plastic syringes preloaded with the following anticoagulant: 38 mmol/L citric acid, 75 mmol/L sodium citrate, 136 mmol/L dextrose, 6 mmol/L EDTA, 6 mmol/L adenosine, and 25 U/mL heparin. The ratio of anticoagulant to blood was 0.2:1.0 (vol/vol). For the prothrombin fragment assay, an anticoagulant containing a synthetic thrombin inhibitor, EDTA and aprotinin was purchased from Byk-Sangtec (Dietzenbach, Germany) and the ratio of anticoagulant to blood was 0.1:0.9 (vol/vol). For fibrinopeptide A assay, an anticoagulant provided by the manufacturer of the assay kit (see below) and containing unspecified amounts of heparin and aprotinin was used (anticoagulant/blood ratio: 0.2:1.0). All of the remaining coagulation and fibrinolysis assays were carried out on blood collected in sodium citrate at a final concentration of 3.8% (wt/vol). All plasma specimens were centrifuged at 4°C for 20 minutes at 2,000g and stored at -80°C until assayed within 2 to 3 months. Assays of the factor IX and X activation peptides and the prothrombin fragment 1+2 were carried out in Boston, MA. All other assays were done in Milan, Italy.

Assays of coagulation activation markers. Activated factor VII (factor VIIa), the enzymatic form of factor VII, was measured by a one-stage, prothrombin time-based assay using a truncated soluble form of recombinant tissue factor (kindly supplied by Dr Y. Nemer son, Mount Sinai Hospital Medical School, New York, NY) that, upon relipidation, reacts with factor VIIa but not with one-chain factor VII.⁹ The factor X activation peptide is cleaved from factor X when this zymogen is activated by factor VIIa-tissue factor complex or by activated factor IX-activated factor VIII-activated surface complex; the factor IX activation peptide is cleaved from factor IX when this zymogen is activated by factor VIIa-tissue factor or activated factor XI. Both peptides were then measured by double-antibody radioimmunoassay (RIA) in plasma extracted before assay to eliminate nonspecific interference from other plasma proteins.^{5,6} Prothrombin fragment 1+2 is a measure of the cleavage of prothrombin by activated factor X (factor Xa) being released from the aminoterminal portion of the molecule during its conversion to thrombin. It was measured in plasma by a previously described double-antibody RIA.¹⁰ Thrombin-antithrombin complex, a measure of thrombin generation and neutralization by antithrombin, was assayed by enzyme-linked immunosorbent assay (ELISA) using a kit from Behringwerke (Marburg, Germany). Fibrinopeptide A is a measure of the formation of fibrin being released from the α -chain of fibrinogen when thrombin converts fibrinogen to fibrin. It was assayed by ELISA in plasma extracted twice with bentonite to remove fibrinogen, using a kit from Boehringer Biochemia (Mannheim, Germany).

Assays of coagulation factors. The coagulant activities of factors II, VII, VIII, IX, and X were measured by standard one-stage functional assays using congenitally deficient plasmas containing less than one percent coagulant activities. Values were expressed in percent of pooled normal plasma. Plasma fibrinogen was measured by a fibrin polymerization assay (Boehringer Biochemia).

Assays of anticoagulant proteins. Antithrombin was assayed by an amidolytic method using the chromogenic substrate S-2238 assembled into a kit by Chromogenix (Molndal, Sweden). Protein C was determined by an amidolytic method (Chromogenix) and total protein S antigen by ELISA (Diagnostica Stago, Asnieres, France), according to the recommendations of the manufacturers of the corresponding assay kits. Tissue factor pathway inhibitor activity was measured in a two-stage chromogenic assay, according to the method of Sandset et al.¹¹

Fibrinolysis assays. Plasminogen and α_2 -antiplasmin were assayed by an amidolytic method using commercial kits (Chromogenix). D-dimer and plasmin-antiplasmin complex were measured

by ELISA (Dimertest, Agen Biomedical, Brisbane, Australia; Behringwerke, Marburg, Germany). Plasminogen activator inhibitor type 1 activity was measured by a chromogenic method (Biopool, Umea, Sweden).

Statistical analysis. The values obtained in controls and centenarians were analyzed after transformation into logarithms, but for descriptive purposes are given as geometric means and 95% confidence intervals. Comparisons between the groups were made by analysis of variance and between-group differences assessed with the Scheffe test. Because multiple comparisons were done in the analysis, the level of statistical significance was set at less than .01. Correlation coefficients were calculated from log-transformed data by linear regression analysis.

RESULTS

Table 1 summarizes the overall results (geometric means and 95% confidence intervals) of the measurements of coagulation and fibrinolysis obtained in centenarians and in the two control populations of younger and older healthy adults. The reference values for each measurement are also provided, reflecting the ranges established in the two laboratories that had tested larger groups of healthy individuals at the time of the standardization of the assays.

Coagulation activation markers. The older controls tended to have higher values than younger controls for all of the measurements, but no difference reached statistical significance ($P > .01$). The plasma concentrations of factor VIIa were significantly elevated in the plasma of centenarians ($P < .01$) (Table 1), although there was overlap with the values measured in both younger and older controls (Fig 1). The plasma levels of the factor X and factor IX activation peptides were very elevated in centenarians ($P < .001$) (Table 1), with little overlap with the control groups, particularly for the factor X activation peptide (Fig 1). The prothrombin fragment 1+2, thrombin-antithrombin complex and fibrinopeptide A were also very elevated in centenarians ($P < .001$) (Table 1 and Fig 2).

There were positive correlations between the levels of the factor IX and factor X activation peptides and between either of them and factor VIIa levels (Table 2). The levels of factor VIIa and of the factor X activation peptide were correlated with those of the prothrombin fragment 1+2 (Table 2). There were no significant correlations between the plasma concentrations of activation markers and creatinine, blood urea, and results of liver function tests, such as albumin and aminotransferases (not shown).

Coagulation factors. Plasma levels of fibrinogen, factor VII, and factor VII tended to be higher in older controls, but the differences from younger controls were statistically significant only for factor VIII (Table 1). Fibrinogen and factor VIII were significantly higher in centenarians than in either control group (Table 1). The concentrations of factor II (prothrombin), factor IX, and factor X were not significantly different or were lower than those found in the control groups (Table 1).

Anticoagulant proteins. In centenarians, the two components of the activated protein C anticoagulant pathway that are measurable in plasma (protein C and protein S) did not differ from either control group (Table 1). The tissue factor pathway inhibitor, the main regulator of the tissue factor/

Table 1. Geometric Means (95% Confidence Intervals) of Hemostasis Measurements in Younger Controls, Older Controls, and Centenarians

Measurements	Younger Controls (1)	Older Controls (2)	Centenarians (3)	P Values			Reference Values
				1 v 2	1 v 3	2 v 3	
Coagulation enzymes and activation peptides							
Factor VIIa (ng/mL)	3.18 (2.76-3.64)	3.24 (2.86-3.67)	4.46 (3.91-5.06)	NS	.002	.004	2.3-5.9
Factor IX activation peptide (pmol/L)	164 (144-185)	207 (187-229)	391 (351-435)	NS	<.001	<.001	131-284
Factor X activation peptide (pmol/L)	86 (81-92)	99 (85-115)	248 (226-272)	NS	<.001	<.001	39-112
Prothrombin fragment 1+2 (nmol/L)	0.56 (0.49-0.64)	0.69 (0.61-0.78)	1.15 (0.93-1.43)	NS	<.001	<.001	0.40-1.30
Thrombin/antithrombin complex (ng/mL)	2.0 (1.6-2.4)	2.4 (2.0-2.8)	5.9 (4.6-7.6)	NS	<.001	<.001	1.0-4.0
Fibrinopeptide A (nmol/L)	0.89 (0.74-1.06)	1.22 (1.00-1.50)	1.99 (1.72-2.31)	NS	<.001	<.001	0.5-2.0
Coagulation factors							
Fibrinogen (mg/dL)	263 (244-284)	312 (287-338)	347 (313-385)	NS	<.001	.005	193-365
Factor VIII (%)	97 (90-105)	134 (119-151)	165 (147-185)	<.001	<.001	.005	53-152
Prothrombin (%)	104 (99-108)	98 (95-102)	88 (82-93)	NS	<.001	.005	71-125
Factor VII (%)	99 (93-105)	110 (104-116)	111 (103-119)	NS	NS	NS	70-133
Factor IX (%)	96 (93-100)	104 (95-113)	107 (98-117)	NS	NS	NS	69-125
Factor X (%)	103 (97-108)	96 (91-102)	83 (77-89)	NS	<.001	.003	72-119
Anticoagulant proteins							
Antithrombin (%)	98 (95-101)	97 (91-104)	91 (85-98)	NS	NS	NS	80-120
Protein C (%)	87 (82-92)	88 (83-93)	90 (83-98)	NS	NS	NS	67-129
Tissue factor pathway inhibitor (%)	95 (89-102)	110 (102-118)	109 (100-118)	NS	NS	NS	61-140
Protein S (%)	106 (98-113)	115 (100-132)	109 (97-123)	NS	NS	NS	65-129
Fibrinolysis measurements							
Plasminogen (%)	97 (93-102)	93 (89-97)	98 (94-102)	NS	NS	NS	70-133
Plasminogen activator inhibitor type I (IU/mL)	3.0 (1.4-6.7)	3.4 (2.3-4.9)	3.4 (1.9-6.0)	NS	NS	NS	2-14
α_2 -antiplasmin (%)	90 (87-93)	94 (90-98)	90 (85-95)	NS	NS	NS	71-125
Plasmin-antiplasmin complex (ng/mL)	295 (262-332)	359 (316-408)	828 (738-927)	NS	<.001	<.001	80-280
D-dimer (ng/mL)	29 (24-36)	50 (39-64)	323 (255-408)	.004	<.001	<.001	2.0-14.0

Abbreviation: NS, not significant.

factor VIIa pathway, and antithrombin, the main inhibitor of thrombin, were also not different (Table 1).

Fibrinolysis measurements. Plasminogen, the key zymogen of the fibrinolytic system, and the two principal inhibitors of the system (plasminogen activator inhibitor type I and α_2 -antiplasmin) were not elevated in centenarians (Table 1). However, centenarians had clear signs of hyperfibrinolysis, expressed by very high levels of D-dimer (an index of plasmin activity on cross-linked fibrin) and plasmin-antiplasmin complex (an index of plasmin formation and neutralization) (both $P < .001$ compared with either control group) (Table 1 and Fig 3). The latter two measurements were positively correlated with each other and with the indexes of thrombin generation and activity (Table 2).

DISCUSSION

A large proportion of centenarians had laboratory signs of coagulation activation, expressed by high levels of enzymes, activation peptides, and enzyme-inhibitor complexes. Procoagulant proteins, such as fibrinogen and factor VIII, that epidemiologic studies have shown to be useful predictors of

cardiovascular disease in middle-aged individuals,^{12,13} were also elevated in centenarians. Yet, these individuals were healthy and their striking biochemical abnormalities were not associated with a disease state, even though the presence of asymptomatic atherosclerosis cannot be ruled out.

Elevated plasma concentrations of markers of coagulation activation might be caused by increased production or by decreased clearance. In their study of age-related changes in these markers, Bauer et al⁴ directly measured the half-life of the prothrombin activation fragment after its infusion into 10 elderly men and showed that the clearance of this moiety was normal. Because these studies could not be performed in our centenarians, decreased clearance of activation markers cannot be directly excluded. On the other hand, centenarians had normal liver and kidney function tests, so that a mechanism of decreased clearance due to liver or renal insufficiency does not appear to be a likely explanation for the high plasma concentrations of these markers.

The most striking finding of this study was that centenarians had very high plasma concentrations of the activation peptide of factor X, the coagulation zymogen placed at the junction of the extrinsic and intrinsic pathways, which indi-

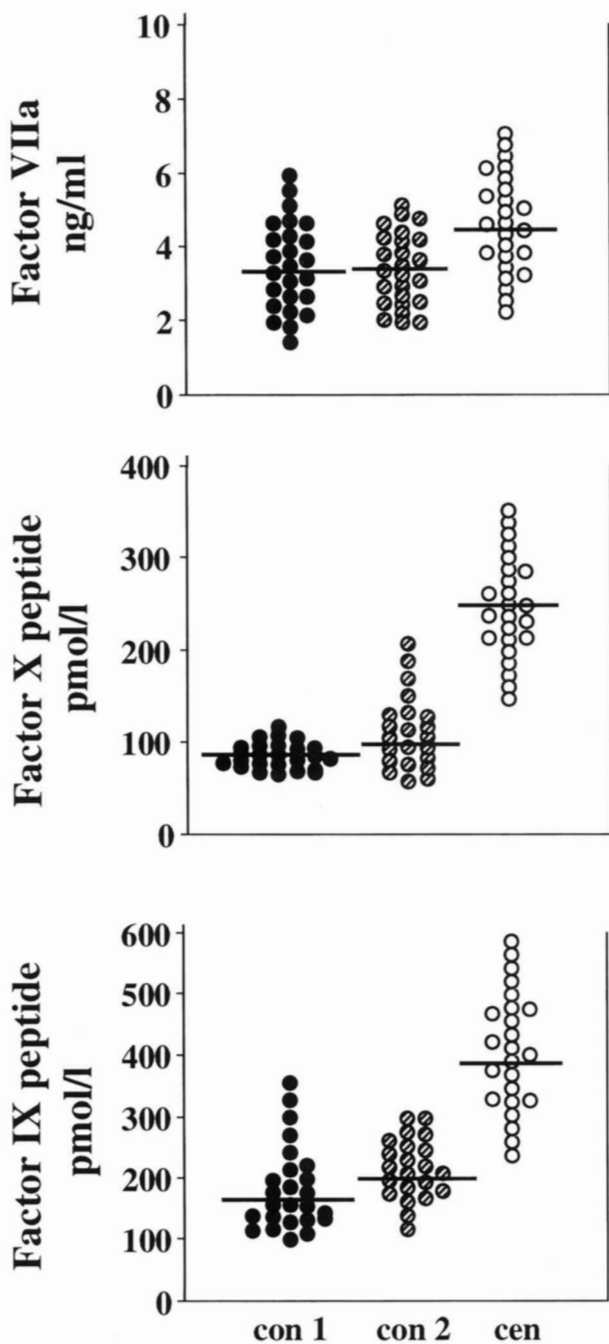


Fig 1. Plasma concentrations of activated factor VII, factor X activation peptide, and factor IX activation peptide in 25 centenarians (cen, ○), 25 controls aged 51 to 69 years (con 2, ○), and 25 controls aged 18 to 50 years (con 1, ●). The solid horizontal lines denote the geometric mean values of each parameter in each group.

cate excessive generation of factor Xa under in vivo conditions. The levels of factor X activation peptide were as high as those found in patients with disseminated intravascular coagulation.⁵ In normal individuals, when there is no pathologic trigger of blood coagulation and no need for enhanced hemostasis, generation of factor Xa occurs predominantly

through the enzymatic action of the factor VIIa-tissue factor complex, which activates factor X.^{14,15} The factor VIIa-tissue factor pathway is also largely responsible for the activation of factor IX.¹⁴ In centenarians, the plasma levels of the factor IX activation peptide were very high, even higher than those found by Bauer et al,⁶ using the same assay method, in elderly individuals aged 71 to 80 years (mean values: 391

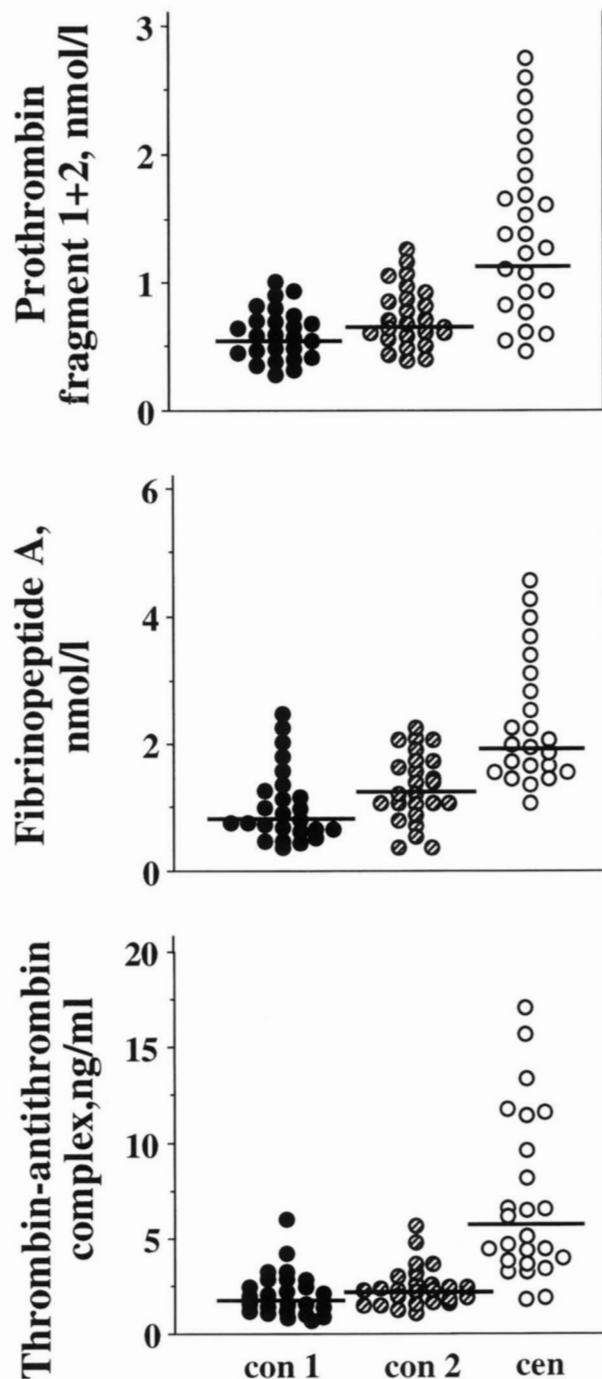


Fig 2. Plasma concentrations of prothrombin fragment 1 + 2, fibrinopeptide A, and thrombin-antithrombin complex. See Fig 1 for symbols.

Table 2. Correlation Coefficients (r values) Between Markers of Coagulation and Fibrinolysis Enzyme Activity in Centenarians

	FXP	FIXP	F1+2	FPA	TAT	D-D	PAP
Factor VIIa (FVIIa)	0.55*	0.45†	0.60*	0.35	0.38	0.29	0.35
Factor X activation peptide (FXP)		0.62‡	0.66‡	0.44†	0.52*	0.38	0.45†
Factor IX activation peptide (FIXP)			0.34	0.41†	0.35	0.29	0.29
Prothrombin fragment 1+2 (F1+2)				0.47†	0.51*	0.51*	0.46†
Fibrinopeptide A (FPA)					0.58*	0.47†	0.34
Thrombin-antithrombin complex (TAT)						0.46†	0.44†
D-dimer (D-D)							0.54*
Plasmin-antiplasmin complex (PAP)							

* $P < .01$.† $P < .05$.‡ $P < .001$.

pmol/L in centenarians v 274 in 71- to 80-year-old individuals). In centenarians, there was a positive correlation between the elevated concentrations of factor X activation peptide and those of the factor IX activation peptide, suggesting that they are associated phenomena. Therefore, it is likely that both are the results of the heightened enzymatic activity of factor VIIa-tissue factor complex, as in physiologic conditions. However, the elevations of factor VIIa concentrations

were less marked than those of the two activation peptides and the correlations weaker. Because factor VIIa has a high affinity for its cofactor tissue factor expressed on cell surfaces,¹⁶ the measurement of the levels of this enzyme in plasma may reflect only a small portion of the enhanced activity of the factor VIIa-tissue factor complex exerted mainly at the level of the cell surfaces.

Consistent with the findings of heightened factor X activation, the late reactions of blood coagulation were also activated in centenarians. Prothrombin fragment 1+2, the product of prothrombin cleavage by factor Xa, was markedly elevated. It is impossible to compare the high values of this fragment in centenarians with those found by Bauer et al⁴ in younger individuals aged 71 to 80 years because the current assay uses a different antibody that gives lower values in healthy individuals. There were also signs of enhanced thrombin generation (high thrombin-antithrombin complexes), fibrin formation (high fibrinopeptide A), and hyperfibrinolysis (high D-dimer and plasmin-antiplasmin levels), which were probably the expression of excessive fibrin formation and dissolution with values similar to those usually found in patients with disseminated intravascular coagulation.

In an attempt to explain mechanistically the dramatic activation of the coagulation system found in centenarians, we surmise that in these individuals there is an enhanced expression and availability of tissue factor, which would, in turn, augment factor VII activation. Tissue factor is usually not expressed in the intact vasculature,¹⁶ but perhaps changes associated with aging can modify the physiologic situation, induce the expression of tissue factor, and thereby accelerate the factor VIIa/tissue factor pathway. The natural regulatory mechanisms may thus be overwhelmed, leading to a state of marked coagulation system activation. The nature of these changes can only be speculative at the moment. Tissue factor expression can be induced by a variety of agonists, such as endotoxins, inflammatory cytokines, activated lymphocytes, growth factors, and oxidized low-density lipoproteins.¹⁶ It has been shown that in centenarians there is a marked increase in the number of circulating activated T lymphocytes (CD3 and HLA-DR positive),¹⁷ which may interact with macrophages-monocytes and/or endothelial cells and upregulate the expression of tissue factor in these cells.¹⁸ This mechanism for enhanced tissue factor expression has been

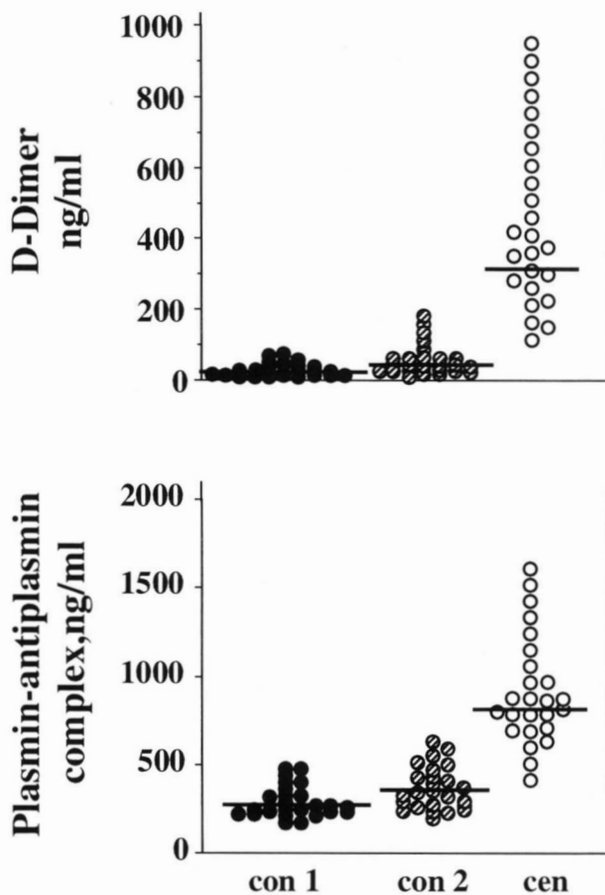


Fig 3. Plasma concentrations of D-dimer and plasmin-antiplasmin complex. See Fig 1 for symbols.

documented to cause the hypercoagulable state associated with unstable angina.¹⁹

Despite high coagulation enzyme activity leading to discrete fibrin formation and secondary hyperfibrinolysis, centenarians were healthy and had no current or past thrombotic episodes. In general, the clinical significance and predictive value of biochemical markers of hypercoagulability remain to be established and are currently being evaluated in prospective studies carried out in middle-aged individuals. Because this investigation shows that marked alterations of these markers are compatible with health and longevity, plasma levels of coagulation activation markers in a study population must always be interpreted in concert with an appropriate control group, and high levels do not necessarily mirror a high risk of arterial or venous thrombosis. The same reasoning can be applied to procoagulant proteins, such as factor VIII and fibrinogen, which are predictors of cardiovascular disease in middle-aged individuals and yet are elevated in healthy centenarians.

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