Menopausal status associated with increased inhibition of blood coagulation

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Postmenopausal women receiving estrogen replacement therapy (ERT) are not as prone to inappropriate venous and arterial thrombosis as are younger women taking oral contraceptives. To establish whether menopausal status per se has any effect on the coagulation-fibrinolytic system normal premenopausal women (mean age 29 years) were compared with younger (mean age 32) and older (mean age 51) surgically menopausal women and a group of naturally postmenopausal women (mean age 53). The results show that in postmenopausal women, irrespective of age or type, the shift is away from clot formation and toward clot inhibition and fibrinolysis as determined by static in vitro analysis. This was characterized by statistically significant increases in antithrombin III antigen, α_1-antitrypsin antigen, and plasminogen activity. These changes may help to explain in part why ERT does not appear to cause increased thrombosis in older women. (AM. J. OBSTET. GYNECOL. 141:149, 1981.)

ELDERLY WOMEN are thought to be at higher risk of thromboembolic disease than younger women.[1] Because changes in the blood procoagulants and inhibitors may cause or contribute to this, the effect of advancing age on coagulation has been studied. Some investigators have found that platelet adhesion and aggregation increase with age, suggesting a tendency toward thrombus formation.[1] Measurements have also been made of the clotting factors in the coagulation system. Most consistently, factors V, VII, XI, and XII and fibrinogen have been reported to increase, whereas other factors are variable.[2-5] Fibrinolytic activity has been shown to decrease with age but has also been reported to be unchanged or increased in other studies.[4-6] These changes may suggest a shift in the procoagulant-inhibitor balance toward thrombus formation; however, these static laboratory changes may represent epiphonomena having little to do with the basic aging process.[7]

The predisposition to thrombosis and changes in coagulation seen in aging women may be the result of the aging process in general or may stem from changes brought about by the altered hormonal status of postmenopausal women. Studies in aged populations do not distinguish between these two effects. The hormonal milieu of premenopausal women is felt by some to provide protection against vascular disease.[8] Women who undergo premature menopause, either surgically or naturally, may be at increased risk of arteriovenous thromboembolic disease.[9-11] Others have noted that estrogen replacement does not appear to increase thromboembolic phenomena in the menopausal group.[12] Menopause therefore may alter thrombogenic tendencies independent of age. In our study we look at the separate effects of menopause and aging on coagulation.

Material and methods

Group selection and composition. Four groups of women were compared. The first group was composed of premenopausal women from the University of

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Table I. Hemostatic determinants in premenopausal and postmenopausal women

	No. of patients	*Mean age (yr)*	*Platelet count**	*PT†*	*Activated PTT†*	*TT†*	*Fibrinogen antigen‡*	*Fibrinogen, clottable§*
Premenopausal women	13	29						
Mean			250,000	0.96	1.25	1.08	260.5¶	300.4‖
SD			±68,000	±0.11	±0.21	±0.61	±108	±58
Surgical menopause <40	23	32						
Mean			251,000	0.97	1.26	0.89	310.5#	362.3
SD			±75,000	±0.06	±0.06	±0.17	±69	±101
Surgical menopause >40	15	51						
Mean			273,000	0.95	1.16	0.84	346.0#	374.8
SD			±80,000	±0.09	±0.14	±0.17	±63	±50
Natural menopause	18	53						
Mean			286,000	0.96	1.00‖	0.82	362.0#	413.7
SD			65,000	±0.13	±0.11	±0.13	±77	±73
P values for significant variance			NS	NS	<0.0002	<0.0005	<0.01	<0.003

*Normal range 150,000 to 450,000/mm³.
†<1.5 Subject/control.
‡Normal range 200 to 450 mg/dl.
§Normal range 150 to 45 mg/dl.
‖Significantly different from other means in column.
¶Mean of premenopausal group differs significantly with natural menopause and >40 surgical group.
#No significant differences between the means.

Florida Women's Outpatient Clinic. The mean age for the group was 29 years (range 24 to 39 years). None of these women were receiving hormone preparations; all had nonmedicated intrauterine devices (IUDs) in place for contraception. Subjects were screened for major problems, heart disease, obesity, hypertension, and past thromboembolic disease.

The remaining three groups were selected from volunteers who were participants in the Menopause Clinic at the University of Florida Center for Climacteric Studies. One group was composed of women under the age of 40 years who had undergone surgical menopause more than 3 months previous to the study. The mean age for this group was 32 years (range 19 to 39 years). A second group was made up of surgically menopausal women over the age of 40 years. The mean age for this group was 51 years (range 43 to 64 years). The final group was composed of women who had signs and symptoms of natural menopause. The mean age for this group was 53 years (range 48 to 60 years). Plasma follicle-stimulating hormone, luteinizing hormone, estrone, and estradiol levels were confirmatory of postmenopausal status for all three groups. None of the women were receiving hormone preparations at the time of the study, therapy having been suspended at least 4 weeks before start of the study. All were given a screening history and physical examination to rule out serious medical problems, heart disease, hypertension, obesity, and past thromboembolic disease.

Blood samples were obtained at 9:00 AM from a carefully done venipuncture. Platelets were enumerated by a Coulter Counter B (Hialeah, Florida) from ethylenediaminetetraacetic acid (EDTA)–anticoagulated blood. The remaining studies were performed on platelet-free plasma obtained from centrifugation of 9 ml blood mixed with 1 ml of 3.8% sodium citrate solution. The prothrombin time (PT), activated partial thromboplastin time (PTT), and thrombin time (TT) were done by routine methods. Clottable fibrinogen was estimated by the Claus method[13]; fibrinogen antigen was determined by radial immunodiffusion using plates purchased from Calbiochem (San Diego, California); antigenic levels of antithrombin III, plasminogen, α_2-macroglobulin, and α_1-antitrypsin were similarly determined. Antithrombin III and plasminogen activity were determined by fluorometric assays (Protopath, American Dade, Miami, Florida). Fibrin degradation products were assayed by the method of Merskey.[14] The within- and between-assay variables were all within the reported values. The variations for Protopath for antithrombin III is 7%, and for plasminogen 5%. All tests were performed by a technician (L. C.) unaware of the exact nature of the study.

Statistical analysis. Analysis of variance and Duncan's multiple range tests were performed for all variables. Means are reported as significant if $p < 0.05$.

Results

By comparing the premenopausal group and the young surgically menopausal group, the differences attributable to hormonal status can be assessed; and by comparing the young menopausal with the older menopausal group, the effects of chronological age are apparent (Tables I and II).

No significant difference between the means was found for the following variables: platelet count, PT, antithrombin III activity, fibrin degradation products,

Table II. Anticoagulation and fibrinolytic factors in premenopausal and postmenopausal women

	No. of patients	Mean age (yr)	Antithrombin III		Fibrin degradation products‡ ≤1:4 titer	α_2-Macroglobulin antigen§	α_1-Antitrypsin antigen‖	Plasminogen	
			Antigen*	Activity†				Activity¶	Antigen#
Premenopausal women	13	29							
Mean			27.2**	102.7	1:2.3	239.2	209.2††	2.9**	12.5
SD			±7.4	±12.0		±75.7	±35.6	±0.4	±3.4
Surgical menopause <40	23	32							
Mean			32.6	114.6	1:1.6	281.8	257.4	3.4	13.9
SD			±5.3	±11.3		±70.6	±48.9	±0.9	±3.5
Surgical menopause >40	15	51							
Mean			32.4	113.4	1:1.6	255.1	251.0	3.7	14.5
SD			±4.7	±15.8		±86.5	±44.6	±0.9	±2.4
Natural menopause	18	53							
Mean			32.1	109.6	1:1.1	269.5	220.0‡‡	3.5	15.0
SD			±7.8	±15.7		±62.3	±51.8	±0.7	±4.0
P values for significant variance			<0.03	NS	NS	NS	<0.008	<0.03	NS

*Normal range 22 to 39 mg/dl.
†Normal range 80% to 120%.
‡Normal ≤1:4 titer.
§Normal range 175 to 450 mg/dl.
‖Normal range 200 to 400 mg/dl.
¶Normal 3.1 ± 0.7 CTA/ml.
#Normal range 10 to 20 mg/dl.
**Significantly different from other means in column.
††Significantly different with means of the two surgical groups.
‡‡Mean varies significantly with respect to <40 surgical group.

plasminogen antigen levels, and α_2 macroglobulin antigen levels.

A common pattern of variance was found for TT, clottable fibrinogen and fibrinogen antigen levels, antithrombin III antigens, and plasminogen activity. The mean values of the premenopausal women differed significantly from those of the postmenopausal women, but there was no difference between the three postmenopausal groups. The respective factors and degrees of significance were: fibrinogen antigen ($p < 0.01$), fibrinogen activity ($p < 0.003$), antithrombin III antigen ($p < 0.02$), and plasminogen activity ($p < 0.03$). All increased with menopausal status. TT ($p < 0.0005$) decreased in the postmenopausal women.

PTT was significantly decreased in the naturally menopausal group ($p < 0.0002$), but there were no significant differences between the other groups.

The variance pattern of α_1-antitrypsin activity was less clearly defined, with the premenopausal group having significantly lower values ($p < 0.008$) when compared with the two surgical groups but not with respect to the naturally menopausal group. The two surgical groups did not vary with respect to each other, and there was no significant difference between the older surgical group and the naturally menopausal group.

Comment

In our study several static and dynamic parameters of hemostasis were measured. These can be divided into factors favoring coagulation and factors opposed to coagulation, including inhibition and plasminogen. Menopausal status, independent of age, does appear to significantly alter both of these pathways.

Dynamic measurements of blood coagulation include PT and PTT. In our study the PT was unaltered, but the PTT was significantly decreased in naturally menopausal women. The PTT for the naturally menopausal group was well within expected normal limits. Variation of individual coagulant factor levels and activity may still occur with advancing age, but it is doubtful that this plays a biologically important role.[7] Our study would therefore seem to indicate that there is no significant change in the activity of either the intrinsic or extrinsic coagulation pathways with age or menopause.

The TT, another dynamic test of blood coagulation, measures the conversion of fibrinogen to fibrin. Generally it reflects changes in fibrinogen levels, although it can be affected by circulating anticoagulants or the presence of fibrin degradation products.[15] In all three groups of postmenopausal women we found a decrease in TT, which correlates consistently with a rise in fibrinogen levels and fibrinogen activity with menopause. This change with age, as has been reported before by other investigators,[3, 4, 6] was not significant in our study.

The antithrombin III–fibrinolytic system may be more important clinically in assessing prethrombotic tendencies, as these are the natural inhibitors of coagu-

lation. Indeed decreased levels of antithrombin III and plasminogen are associated with increased arterial and venous thrombosis.[16–18] Oral contraceptive administration has been reported to be associated with a decrease in antithrombin III levels and an increase in thromboembolic phenomena.[19, 20] In our study antithrombin III levels and activity were found to increase after menopause, although only the change in antigen was statistically significant. Because the decrease in antithrombin III levels with oral contraceptive use is felt to be an estrogen-dependent effect, the increased levels at menopause may represent release from natural estrogenic inhibition. Two other plasma proteins having antithrombin activity, α_2-macroglobulin and α_1-antitrypsin, were found to be increased in menopausal women, regardless of age, when compared with premenopausal women.

To assess the plasma fibrinolytic system plasminogen activity and antigen were assayed. Plasminogen activity showed a significant increase with menopause. Plasminogen antigen levels showed a similar increase, but this was not found to be statistically significant. This suggests that the postmenopausal status is associated with an increase in potential fibrinolytic activity, although the normal fibrin degradation products show no evidence for increased fibrinolysis.

In general our study shows alterations in postmenopausal women that may result in a shift away from clot formation and toward clot inhibition and fibrinolysis. These changes may help explain why estrogen replacement therapy appears to be less likely to cause increased thrombosis in older women. In premenopausal women the effect of oral contraceptives on increasing thromboembolism is established. The incidence of clinical symptoms has been linked to the dose and preparation of estrogen used.[21] The lack of thrombosis associated with treatment of postmenopausal women was previously thought to be due to the low dose and "natural" estrogen preparations used for replacement therapy, although some researchers believe that "natural" preparations do alter coagulation parameters similar to synthetic preparations.[22, 23] It can be postulated from our results that the loss of endogenous estrogen in the postmenopausal woman creates a shift in hemostasis regulation toward fibrinolysis and clot inhibition making them more resistant to the thrombogenic properties of exogenous estrogens than premenopausal women.

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