*Transdermal Progesterone: Effects on Menopausal Symptoms and on Thrombotic, Anticoagulant, and Inflammatory Factors in Postmenopausal Women

Kenna Stephenson, MD, FAAFP
Pierre F. Neuenschwander, PhD, FAHA
Anna K. Kurdowska, PhD
Barbara Pinson, MS, MD
Carol Price, MSN
The University of Texas Health Science Center at Tyler
Tyler, Texas

Abstract Conventional hormone replacement therapy increases a woman's risk of thrombotic events as evidenced in large prospective clinical trials, including HERS I and the Women's Health Initiative. A possible mechanism for this is the unfavorable net effects of conjugated equine estrogens and medroxyprogesterone acetate on factors involved in hemostatic balance and inflammation. The objective of this study was to examine the short-term effects of transdermal progesterone on menopausal symptoms and serum levels of hemostatic, inflammatory, and immune signaling factors. In a prospective, randomized, double-blinded, placebo-controlled, crossover study, 30 healthy postmenopausal women received either 20 mg/day of transdermal progesterone or placebo for 4 weeks, followed by a 4-week washout period, and were then crossed over to receive either placebo or active drug for an additional 4 weeks. Baseline, 4-week follow-up, and end-of-study values were obtained for the Greene Climacteric Scale, and for serum levels of total factor VII:C, factor VIIa, factor V, fibrinogen, antithrombin III, plasminogen activator inhibitor-1, C-reactive protein, interleukin-6, matrix metalloproteinase-9, and tumor necrosis factor-α. Transdermal progesterone significantly improved Greene Climacteric Scale scores. In sharp contrast to previous studies of conventional hormone replacement therapy, no detrimental effect was observed on any of the hemostatic or inflammatory components examined. Administration of transdermal progesterone at a daily dose of 20 mg significantly relieves menopausal symptoms in postmenopausal women without adversely altering prothrombotic potential. We suggest, therefore, that this treatment be seriously considered as an effective and safe alternative clinical therapy for women suffering from menopausal symptoms.

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*Introduction

In the U.S., 40 million women are currently experiencing menopause and an additional 20 million women will reach menopause during the next decade. Women aged 50 years or older will number more than 1.2 billion worldwide by the year 2030; the aging of the female population and resulting physiological and pathophysiological changes induced by the menopausal transition are of clinical concern.[1] Perimenopausal, menopausal, and postmenopausal women may experience a decline in health-related quality of life due to hot flashes, disruption of sleep, fatigue, and other symptoms associated with menopause. For over five decades, women in the U.S. have used synthetic hormone replacement therapy (HRT), including conjugated equine estrogens and/or progestin, in an effort to relieve menopausal symptoms. Findings of the Women's Health Initiative study (WHI) have brought forth concerns about the safety of these therapies from women and their healthcare providers.[2,3]

Although its has been asserted, on the basis of observational studies, that HRT has cardioprotective properties in postmenopausal women, this observation has not been replicated in large, prospective, double-blind, randomized clinical trials.[2-4] The WHI found that the use of conjugated equine estrogens alone decreased the risk of hip fracture but increased the risk of stroke, probable dementia, and venous embolism, and had no statistically significant impact on coronary heart disease.[2] The use of conjugated equine estrogens in combination with medroxyprogesterone acetate, a synthetic progestin, increased the risk of breast cancer, heart disease, probable dementia, venous thromboembolism, and stroke. From this, the WHI authors concluded that the overall risk exceeded the benefits of HRT.[2,3,5] In light of these conclusions and the known risks of HRT, clinical therapy for menopausal symptoms remains a significant challenge. Women as healthcare consumers are increasingly utilizing transdermal preparations of plant-derived progesterone, yet the effects of progesterone on thrombotic and inflammatory mechanisms have not been identified.

Observational studies, randomized clinical trials, and animal investigations have revealed correlations between levels of hemostatic and inflammatory factors, as well as proinflammatory cytokines, and the risk of cardiovascular disease.[6-8] Prothrombotic risk has been studied in women receiving combinations of conjugated equine estrogens, synthetic estrogen, and synthetic progestins.[9-16] While studies of hemostatic factors during exogenous HRT demonstrate consistent decreases in fibrinogen and plasminogen activator inhibitor-1 (PAI-1) levels that are suggestive of reduced prothrombotic potential, the measured decrease in antithrombin III level and concomitant increases in factor V, factor VII, and factor VIIa levels are more consistent with an increase in prothrombotic potential. While these seemingly conflicting findings are most likely due to genetic variations among individuals that result in disparities in the net effect of estrogen, it is clear that, in the setting of dyslipidemia and obesity, the net effect of HRT is to increase thrombotic risk.[12]

In addition to the aforementioned effects of HRT on hemostatic factors, conjugated equine estrogens and medroxyprogesterone acetate have been shown to increase the level of C-reactive protein (CRP), an inflammatory marker that is considered an independent risk factor for cardiovascular disease in healthy postmenopausal women.[9,17-19] Medroxyprogesterone acetate has been found to have a prothrombotic effect on coronary arteries in animal menopause models using rhesus monkeys and cynamolgus monkeys.[20-22] Moreover, several studies of HRT in postmenopausal women, including the WHI, have demonstrated an associated increase in interleukin-6 (IL-6) level in hormone users.[3,23] IL-6 is a proinflammatory cytokine that, along with tumor necrosis factor-α (TNF-α), has both endocrine and immune functions and has been identified as an important regulator of estrogen synthesis in breast and adipose tissue.[24] Abnormal elevations of IL-6 and TNF-α promote tumor cell growth *in vitro* and *in vivo*.[25] Abnormal elevations of IL-6 have been associated with the pathogenesis of atherosclerosis and coronary artery disease, lipid abnormalities, sleep apnea, frailty, and obesity.[26,27] HRT has been shown to increase level of matrix metalloproteinase 9 (MMP-9) in postmenopausal women, and such elevations are significant because MMP-9 accumulates in atherosclerotic plaques and is thought to contribute to degradation of the extracellular matrix, leading to plaque rupture.[28]

The ESTHER clinical investigations suggested that the delivery mechanism of HRT may be the essential factor in determining thrombotic effects. Transdermal estradiol has been shown to confer lower thrombotic risk than oral conjugated equine estrogens.[29] Because postmenopausal women require endometrial protection, the thrombotic and inflammatory effects of combined progesterone and estradiol must be evaluated. The Writing Group for the WHI concluded that,

> …transdermal estradiol and progesterone, which more closely mimic endogenous hormones when used in replacement therapy, may have more favorable outcomes as compared to conjugated equine estrogens and medroxyprogesterone acetate.[3]

Progesterone was first extracted and used clinically in the 1930s. While compounded plant-derived progesterone is identical in chemical structure to endogenous progesterone, medroxyprogesterone acetate is not. This may account for the statistically significant differences observed in clinical efficacy, tolerability, and physiological activities between progesterone and medroxyprogesterone acetate in clinical investigations.[30,31] Studies have demonstrated the clinical efficacy of progesterone in menopausal symptom relief, infertility treatment, and perinatal therapy, and in producing favorable effects on coronary arteries, endometrium, and breast tissue.[32-36] Progesterone has a significant role in central and peripheral nervous system responses to pain, inflammation, and stress.[37-39] Furthermore, it mediates favorable nongenomic and genomic effects on coronary vasculature and cardiac functions, as demonstrated *in vitro* and in animal studies.[40-43] Studies have demonstrated that topical progesterone has a favorable metabolic effect with respect to its patterns of distribution and metabolism, which are comparable to those reported for endogenous progesterone secretion and intravascularly administered progesterone.[44,45] Transdermal delivery bypasses the first-pass effect and provides a system of constant release, thereby avoiding excessively high peak plasma levels. However, data regarding *in vivo* effects of progesterone on hemostatic factors, inflammatory factors, and

proinflammatory cytokines are lacking. In light of the unfavorable effects of conventional HRT on these factors and the potential benefits of transdermal progesterone in animal models of menopause, we initiated a prospective, blinded, placebo-controlled, crossover study to examine the short-term effects of transdermal progesterone in 30 healthy postmenopausal women. The aim of this study was to measure the specific effects of progesterone on hemostatic and inflammatory factors and Greene Climacteric Scale scores.

*Methods

Study Population

After obtaining permission from the Internal Review Board at our institution, we recruited postmenopausal women from the community. Eligibility criteria included age of at least 30 years and (1) cessation of menstruation for at least 12 months as a result of natural menopause or surgical removal of both ovaries, with or without a hysterectomy, or (2) cessation of ovarian function by chemotherapy or radiation. Exclusion criteria included any recent history of alcohol or drug abuse; any treatment with investigational drugs within 4 weeks prior to study entry; any moderate or severe chronic illness; any current ingestion of prescription or over-the-counter sex steroids, steroid hormones, nonsteroidal anti-inflammatory drugs, anticoagulants, antibiotics, or cholesterol-lowering drugs; or any use of nutritional supplements in excess of United States Recommended Daily Allowances. Subjects were recruited from the East Texas region via newspaper announcements and posted fliers in the health center.

Study Design

Subjects who met the inclusion criteria were enrolled after giving informed consent. Each subject was assigned to one of two study groups using a web-based research randomization program.[46] During the first 4 weeks of the study, group 2 received the active drug (transdermal progesterone 20 mg/day), while group 1 received a placebo. The drug and placebo were each compounded by a trained compounding pharmacist according to U.S. Food and Drug Administration (FDA) guidelines for compounded drugs and using FDA-approved materials and packaging.

The packaging, dose, consistency, and odor of the placebo preparation were matched to those of the active drug preparation by the compounding pharmacist using HRT Base, a lipophilic emulsion-type base manufactured by Professional Compounding Centers of America (Houston, Texas). The initial 4-week treatment period was followed by a 28-day wash-out period, after which group 2 received the placebo and group 1 received the active drug. In both treatment periods, subjects were instructed to apply 1 mL of the transdermal preparation at bedtime to the skin of the neck, chest, breast, inner arm, wrist, or back of hand, and to rotate the site of application so as to apply the transdermal preparation to a different site each day. At the end of each week, subjects returned the syringes that had contained the transdermal compound to the study coordinator. The group assignments were masked so that investigators and research coordinator were unaware of which subjects were receiving active drug.

All subjects underwent an initial medical history and physical examination. Baseline values were obtained for the Greene Climacteric Scale score, and for plasma levels of total factor VII:C, factor VIIa, factor V, fibrinogen, antithrombin III, PAI-1, CRP, TNF-α, MMP-9, and IL-6. Follow-up values were obtained at 4 weeks and 12 weeks, and all subjects underwent an end-of-study physical examination. Weekly telephone interviews were conducted to inquire about any changes in health status, new medication use, and compliance with application of the transdermal preparation.

Three subjects dropped out of the study. One subject was dismissed from the study during week 3 because she ingested prescribed systemic steroids for acute traumatic joint inflammation, and two subjects discontinued the study because they were unable to attend required study clinic visits. No subject discontinued participation because of adverse events or side effects of the study drug.

Measurement of Hemostatic Factors

PAI-1 levels were measured by using the Chromolize PAI-1 assay kit (DiaPharma Group, Inc., West Chester, Ohio), which measures active PAI-1. Assays were done according to the manufacturer's directions, and the standard line was linear out to 50 IU/mL, with a minimal detectable concentration of roughly 2 IU/mL.

Factors V, VII:C, and VIIa, antithrombin III, and fibrinogen were all measured by modified clotting assays that are described separately in this section. The factor-deficient plasmas used in these assays were obtained as congenitally deficient plasmas from George King Biomedical, Inc. (Overland Park, Kansas). The clotting time for each assay was measured at 37°C in duplicate using a Coag-A-Mate XM (BioMérieux, Durham, North Carolina), and each sample was assayed three times on different days. Clotting times were converted into concentrations by comparison to standard curves performed daily for each assay with pooled normal human plasma using at least four different plasma dilutions. All samples were diluted appropriately to obtain clotting times within the standard curve for each respective assay. The concentration obtained was then corrected for the dilution to obtain the actual level present in the sample. Typical dilutions required ranged from 1:10 to 1:75 (indicated). All dilutions were in Tris-buffered saline solution containing 0.1% bovine serum albumin as a carrier.

Factor V: Diluted sample (typically 1:40) was added to an equal volume of factor V–deficient plasma that had been supplemented with 0.5 mg/mL inositihin (Asolectin; Sigma Chemical Co., St. Louis, Missouri). Clotting was initiated after a 1-minute warming period by the rapid sequential addition of equal volumes of purified bovine factor Xa (250 ng/mL; Haematologic Technologies, Inc., Essex Junction, Vermont) and 25 mM $CaCl_2$. When plotted using log-log axes, the standard curve for this assay was linear from 0.3 to 3 nM factor V (using a normal plasma concentration of 30 nM).

Factor VII:C: Diluted sample (typically 1:25) was added to an equal volume of factor VII–deficient plasma and allowed to warm for 1 minute. A bolus of two volumes of thromboplastin reagent (Pacific Hemostasis Thromboplastin-D; Fisher Diagnostics, Middletown, Virginia) was then added to initiate clotting. When plotted on a log-log scale, the standard curve was linear between 2 and 100 ng/mL factor VII (using a normal plasma concentration of 500 ng/mL).

Factor VIIa: The assay for factor VIIa was done essentially as described elsewhere.[47] In short, diluted sample (typically 1:10) was added to sTF reagent and allowed to warm for 1 minute. Clotting was then initiated by the addition of an equal volume of 25 mM $CaCl_2$. The sTF reagent comprised 1 mcMol/L soluble tissue factor, 200 mcMol/L phospholipid vesicles, and 0.1% bovine serum albumin in Tris-buffered saline. The soluble tissue factor used was bacterially expressed and prepared as described elsewhere.[48] Purified phospholipids were obtained from Avanti Polar Lipids, Inc. (Alabaster, Alabama), and phospholipid vesicles composed of 40 mol% phosphatidylethanolamine, 40 mol% phosphatidylcholine, and 20 mol% phosphatidylserine were prepared by sonication to obtain small unilamellar vesicles. The standard line for this assay was prepared by using purified human factor VIIa from Haematologic Technologies, Inc. When plotted on a log-log scale, this standard line was linear between 0.03 and 30 ng/mL factor VIIa.

Fibrinogen: Diluted sample (typically 1:10) was prewarmed for 1 minute before the addition of 0.33 volume of 50 U/mL topical bovine thrombin (Thrombin-JMI; Jones Pharma, Inc., St. Louis, Missouri) to initiate clotting. When plotted on a log-log scale, the standard line was linear between 0.08 and 0.6 mg/mL fibrinogen (using a normal plasma concentration of 3 mg/mL).

Antithrombin III: Diluted sample (typically 1:75) was prewarmed for 1 minute before the addition of 1 U/mL thrombin (topical bovine thrombin). For this assay, the sample dilution buffer also contained 0.6% polyethylene glycol 8000 and 1.5 U/mL heparin (Grade IA from porcine intestine; Sigma). After a 30-second incubation, 0.33 volume of purified fibrinogen 8 mg/mL (from bovine plasma; Sigma) was added to initiate clotting. This assay measures functional antithrombin III and is based on the ability of the heparin-antithrombin III complex to proportionally inhibit thrombin that has been added in excess. When plotted on a log-log scale, the standard curve obtained was valid between 50 and 125 nM antithrombin III (using a normal plasma concentration of 5 mcMol/L).

Measurement of Inflammatory Factors

CRP levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Life Diagnostics, Inc., West Chester, Pennsylvania) according to the manufacturer's instructions. The minimum detectable concentration of CRP in this assay was 0.1 mg/L.

Measurement of Cytokines

Plasma concentrations of human TNF-α and IL-6 were measured using ELISA kits (CytoSets, Biosource, Camarillo, California) specific for each cytokine according to manufacturer's recommendations. The sensitivity of both assays was approximately 15 pg/mL.

Measurement of Matrix Metalloproteinase-9

Plasma concentrations of MMP-9 were measured by ELISA assay. The specific methodology was that recommended by the manufacturer (Calbiochem, San Diego, California).

Measurement of the Greene Climacteric Scale Score

Permission was obtained for use of this standardized questionnaire, which has been shown to have internal and external validity in the quantitative assessment of menopausal symptoms. The scale is a standardized 4-point ordinal scale of 21 questions based on factor analysis studies of perimenopausal and menopausal symptoms affecting quality of life. These factors included vasomotor, somatic, and psychological factors divided into moods of anxiety or depression. The Greene Climacteric Scale was developed to provide a standard measure of common climacteric symptoms experienced by the

majority of menopausal women.[49] This scale is psychometrically sound and has high content validity and test-retest reliability.

Statistical Analyses

Levels of hemostatic factors and inflammatory factors were compared between groups by using the one-way analysis of variance. The nonparametric Mann-Whitney test was used for data sets with widely variable distributions, and the Student's *t*-test was used for multiple group analyses. All results are reported as mean ± standard deviation, and $P < 0.05$ was considered significant. Statistical calculations were performed by using SIGMASTAT (SPSS Science Inc, Chicago, Illinois). Demographic characteristics of the two groups were compared by using Lavene's test for equality of variances. Greene Climacteric Scale data were analyzed by using the Wilcoxon test.

*Results

A total of 30 women were recruited into this study. Subjects ranged in age from 43 to 74 years, with a combined median age of 57 years. Participants were randomly assigned to group 1 or group 2. The mean ages were 60 years for group 1 and 53 years for group 2. Body mass index as calculated for all subjects ranged from 21 to 38, with median values of 28.31 for group 1 and 26.69 for group 2. All subjects had a college education; 28 of 30 subjects were of Caucasian ethnicity, one was Native American, and one African American. Of the 30 subjects, 26 had a remote history of HRT, including conjugated equine estrogens, medroxyprogesterone acetate, synthetic estrogens, and/or other synthetic progestins. Alcohol consumption at a frequency of five or fewer alcoholic beverages per month was reported by 61.5% of both groups, and the remaining 38.5% reporting no alcohol consumption. Tobacco use was reported by 7.7% of group 1 and 30.8% of group 2.

Although no adverse events were reported by any of the subjects during weekly interviews, a few subjects reported side effects. One subject reported 3 days of light menstrual spotting, which end-of-study analysis indicated occurred while using the progesterone cream. A second subject reported dizziness when applying the cream

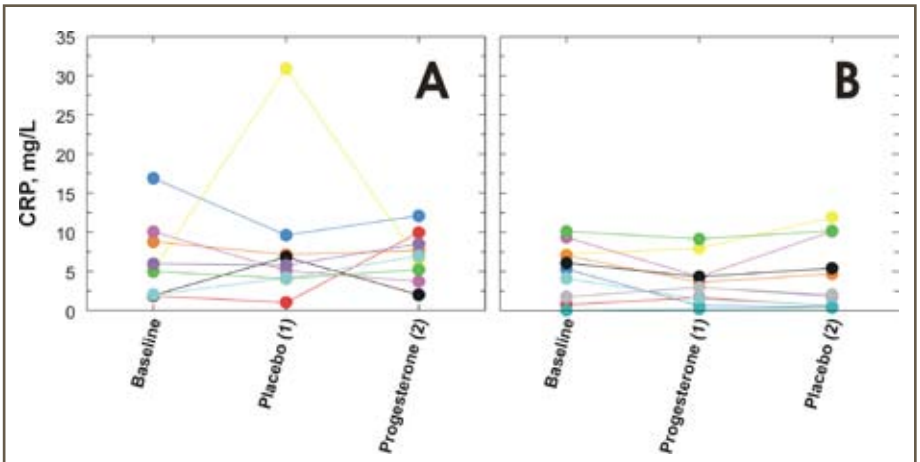

FIGURE 1. CRP levels in study individuals. (A) Group 1; subjects receiving placebo followed by progesterone. (B) Group 2; subjects receiving progesterone followed by placebo.

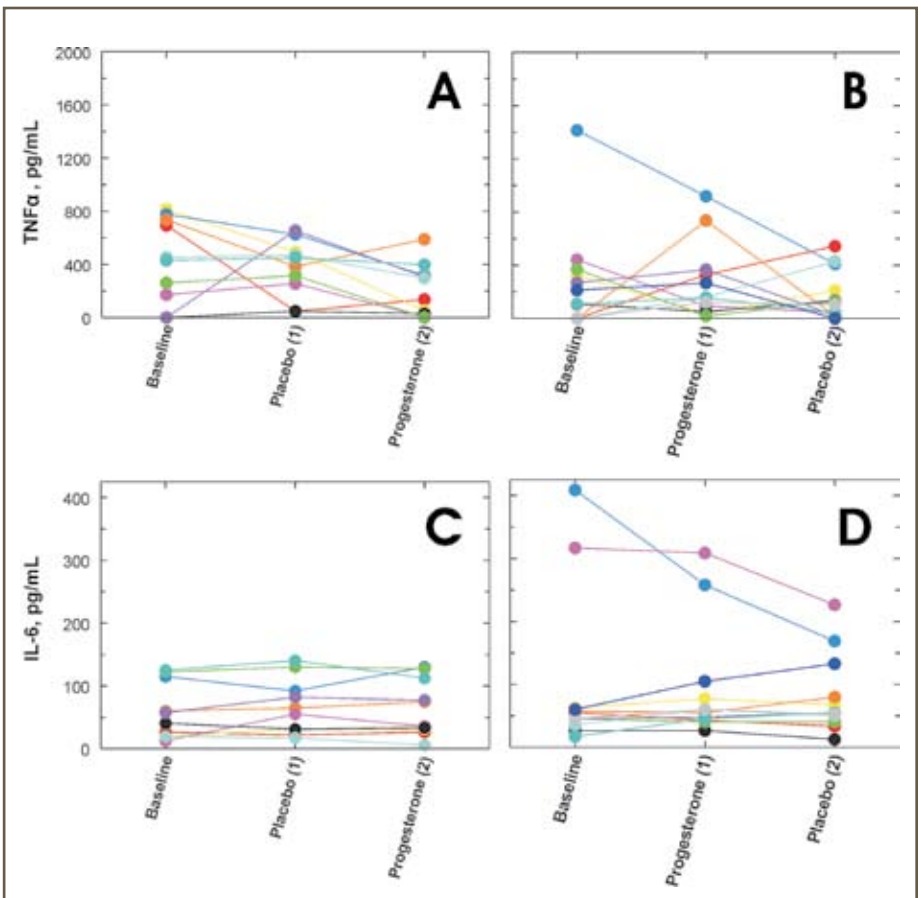

FIGURE 2. TNF-α levels (top panels) and IL-6 levels (bottom panels) in study individuals. Panels A and C are group 1 individuals and panels B and D are group 2 individuals.

to the neck area. Analysis indicated that this occurred while the subject was receiving placebo. A third subject reported a mild headache following cream application to the neck area. Analysis indicated that this occurred while using the progesterone cream. Progesterone levels were monitored during treatment with both the placebo and progesterone, and results demonstrated subject compliance with application of the transdermal progesterone.

There were no statistically significant measurable changes in inflammatory or hemostatic factors with the application of topical progesterone cream. Baseline levels of CRP were 6 ± 5 mg/L for group 1 and 5 ± 3 mg/L for group 2. Follow-up CRP level ranges were 8 ± 9 mg/L for placebo and 7 ± 3 mg/L for progesterone in group 1, and 4 ± 3 mg/L for progesterone and 4 ± 4 mg/L for placebo in group 2. The large standard deviations of these group ranges is a result of the wide variation among individuals (Figure 1) and is not indicative of poor assay sensitivity.

Baseline levels of TNF-α and IL-6 were 434 ± 316 pg/mL and 60 ± 45 pg/mL, respectively, for group 1, and 271 ± 393 pg/mL and 98 ± 126 pg/mL, respectively, for group 2. Posttreatment levels of TNF-α were 374 ± 211 pg/mL for placebo and 213 ± 199 pg/mL for progesterone in group 1, and 271 ± 285 pg/mL for placebo and 174 ± 186 pg/mL for progesterone in group 2. For IL-6, the values were 67 ± 44 pg/mL for placebo and 66 ± 45 pg/mL for progesterone in group 1, and 94 ± 91 pg/mL for placebo and 81 ± 63 pg/mL for progesterone in group 2 (Figure 2).

Factor V levels at baseline were 29 ± 7 nM for group 1 and 27 ± 7 nM for group 2, which compares well with expected normal concentrations in plasma (30 nM). Ranges of factor V level were 28 ± 5 nM for placebo and 31 ± 8 nM for progesterone in group 1, and 29 ± 7 nM for placebo and 29 ± 7 nM for progesterone in group 2. No significant differences were observed for individuals (Figure 3).

Total factor VII:C levels at baseline were 497 ± 71 ng/mL for group 1 and 447 ± 95 ng/mL for group 2, excluding an outlier (Figure 4B). The expected normal concentration of factor VII:C in plasma is 500 ng/mL. Ranges of factor VII:C were 502 ± 93 ng/mL for placebo and 555 ± 167 ng/mL for progesterone in group 1, and 533 ± 98 ng/mL for placebo and 455 ± 88 ng/mL for progesterone in group 2 (excluding outlier). Once again, no significant differences were observed for individuals (Figure 4A and 4B). A similar lack of effect was observed on activated factor VII (factor VIIa) levels. Baseline levels of factor VIIa were 2.3 ± 0.5 ng/mL for group 1 and 2.1 ± 0.8 ng/mL for group 2, excluding the outlier (Figure 4D). This correlates well with the reported range of factor VIIa concentrations in normal plasma: 0.5 to 8.5 ng/mL with a mean of 3.6 ng/mL.[47] Measured ranges of factor VIIa were 2.5 ± 0.9 ng/mL for placebo and 2.6 ± 0.9 ng/mL for progesterone in group 1, and 2.7 ± 1.5 ng/mL for placebo and 2.2 ± 1.1 ng/mL for progesterone in group 2. No significant differences were observed for individuals (Figure 4C and 4D).

Fibrinogen baseline levels were 3.2 ± 0.6 mg/mL for group 1 and 3.5 ± 0.9 mg/mL for group 2 (compared to a value of 3.0 mg/mL for normal plasma). Measured ranges of fibrinogen were 3.7 ± 0.7 mg/mL for placebo and 3.4 ± 0.4 mg/mL for progesterone in group 1, and 3.4 ± 0.5 mg/mL for placebo and 3.9 ± 0.5 mg/mL for progesterone in group 2, with no significant differences for individuals (Figure 5).

Antithrombin III levels at baseline were 5.6 ± 0.9 mcMol/L for group 1 and 5.6 ± 0.9 mcMol/L for group 2 (compared to a value of 5 mcMol/L in normal plasma). Ranges of antithrombin were 5.3 ± 1.0 mcMol/L for placebo and 4.9 ± 1.0 mcMol/L for progesterone in group 1, and 5.5 ± 0.8 mcMol/L for placebo and 5.6 ± 0.6 mcMol/L for progesterone in group 2 (Figure 6A and 6B).

PAI-1 levels at baseline were 3 ± 2 IU/mL, with one outlier at 33 IU/mL, for group 1 and 2 ± 2 IU/mL for group 2 (Figure 6C and 6D). Follow-up levels were 3 ± 4 IU/mL for placebo (outlier at 32 IU/mL) and 12 ± 6 IU/mL for progesterone (outlier at 38 IU/mL) in group 1 and 5 ± 4 IU/mL for placebo and 5 ± 5 IU/mL for progesterone in group 2.

Baseline MMP-9 levels were 78.9 ± 19.7 ng/mL for group 1 and 77.1 ± 12.9 ng/mL for group 2. Posttreatment follow-up levels in group 1 were 67.5 ± 31.1 ng/mL for progesterone and 74.6 ± 14.5 ng/mL for placebo ($P = 0.51$). For Group 2, follow-up values were 81.2 ± 15.6 ng/mL for placebo and 78.8 ± 19.9 ng/mL for progesterone ($P = 0.86$). Results are shown in Figure 7A and 7B.

Significant posttreatment decreases in Greene Climacteric Scale scores were found for group 2 but not for group 1 ($P < 0.05$). Group 2 subjects had higher Greene Climacteric Scale scores for the domains of psychological well-being, depression, and anxiety at baseline (consistent with decreased quality of life).

*Discussion

Transdermal progesterone may be considered an appropriate therapeutic option in postmenopausal women because of its neutral effects on hemostatic and inflammatory factors. In contrast to medroxyprogesterone acetate and conjugated equine estrogens, transdermal progesterone is unlikely to increase the risk of thrombotic events since it appears to cause no changes in these acute phase reactants. These findings are consistent with animal models of menopause which demonstrate that progesterone has a protective effect against drug-induced vasospasm in coronary arteries and vascular smooth muscle cell reactivity.[20-21] Cytokines are increasingly recognized as significant contributors to immune system functions as well as some endocrine functions, and an imbalance of proinflammatory and anti-inflammatory cytokines has been associated with both cardiovascular disease and cancer risks.[24,27] While conjugated equine estrogens and medroxyprogesterone acetate have been demonstrated to increase proinflammatory cytokine levels, progesterone had no effect on measured cytokine levels in this study.[9-12]

In group 2, progesterone application produced significant beneficial changes over placebo ($P < 0.05$) with respect to Greene Climacteric Scale scores for psychological well-being, mood, and symptoms of depression and anxiety. In contrast, no statistically significant effect was observed in Greene Climacteric Scale scores for group 1. One possible explanation for this observed difference may be the disparity in mean age between the two groups, which was statistically significant. As supported by the National Institutes of Health funded Study of Women Across the Nation (SWAN), decreases in estrogen sensitivity of the hypothalamic-pituitary-adrenal axis occur as women age though the menopausal transition, and estrogen levels are erratic and often elevated in younger perimenopausal/menopausal women.[50] In the WHI, the prevalence of symptoms at entry, including

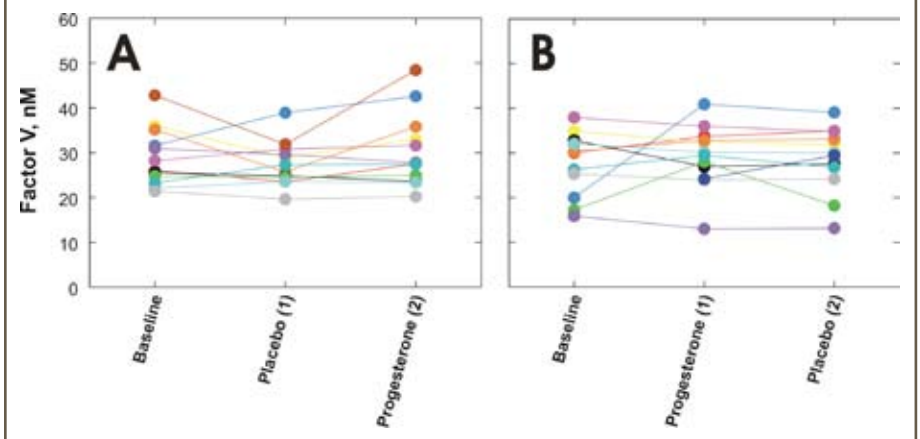

FIGURE 3. Coagulation factor V levels in study individuals. Group 1 is shown in panel A and group 2 in panel B.

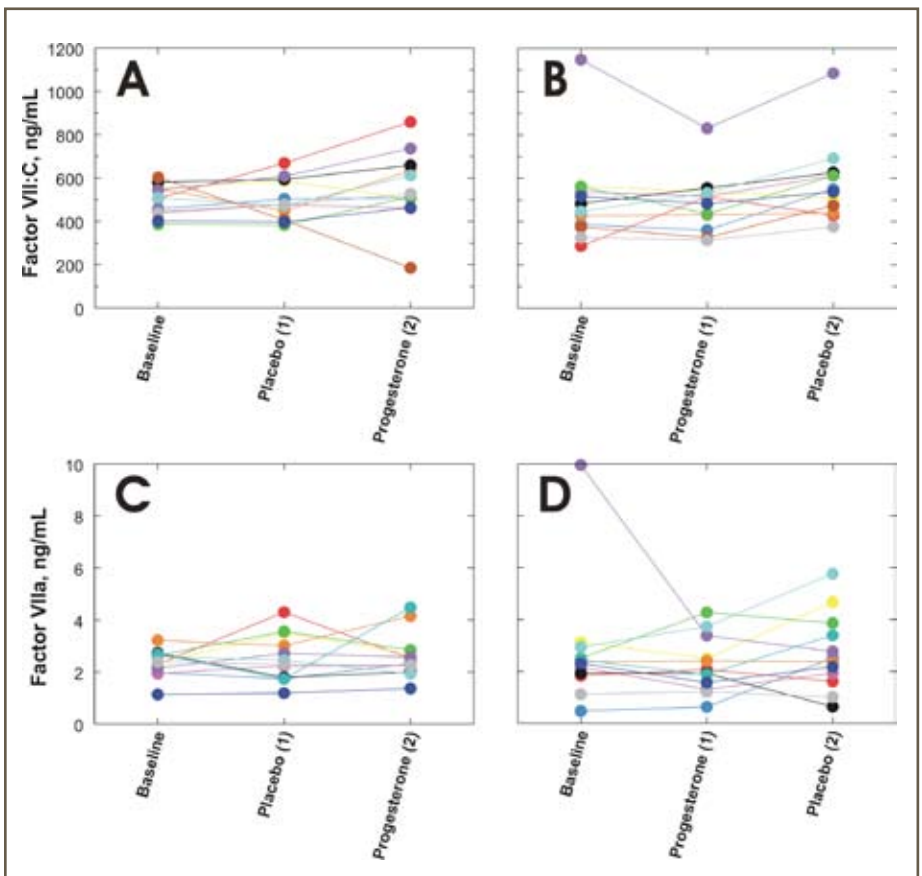

FIGURE 4. Total factor VII:C levels (top panels) and activated factor VII levels (factor VIIa; bottom panels) in study individuals. Panels A and C are group 1 individuals and panels B and D are group 2 individuals.

hot flashes, vaginal dryness, headache, and mood swings, was lower in older subjects.[2,3] Thus, younger women are likely to have more severe symptoms than older women, and, as a result, may have more pronounced therapeutic responses to sex steroids.

SWAN has further characterized the perimenopausal transition as a dysfunction of the hypothalamic-pituitary-ovarian axis with annual declines in luteal function and progesterone levels; thus, it is plausible that progesterone, rather than estrogen, should be considered as initial therapy in symptomatic perimenopausal women.[51] Group 2 also had a higher percentage of subjects who smoked, and smoking has been associated with more pronounced menopausal symptoms. The beneficial effects of progesterone on sleep, cognitive function, irritability, tension, depression, and anxiety in younger subjects supports the neuroactive role of progesterone in its action on gamma-amino-butyric acid receptors in the brain and peripheral nervous system.[52,53] It is conceivable that the significant increase in prescribing of psychotropic agents (including antidepressants, anxiolytics, and hypnotics) for mood disorders in perimenopausal and menopausal women as revealed in epidemiological studies may be related to low progesterone levels rather than to a purely psychiatric etiology.

*Conclusion

Finding therapies to alleviate menopausal symptoms without inducing adverse events or significant side effects is of paramount importance in addressing the needs of an estimated 40 million perimenopausal and menopausal women in the U.S. Increasingly, women present to their healthcare providers with concerns about menopausal symptoms that interfere with daily quality of life and activities, and with requests for "bioidentical" or "natural" hormone therapy.[53] Our study findings support the position that transdermal progesterone is effective in symptom reduction without increasing thrombotic and inflammatory risks. Although it has been reported that transdermal progesterone should be thought of as inducing the same adverse health risks as medroxyprogesterone acetate, our data do not support this with respect to the hemostatic and inflammatory factors examined.[54] Progesterone administered transdermally warrants consideration as a safe and ef-

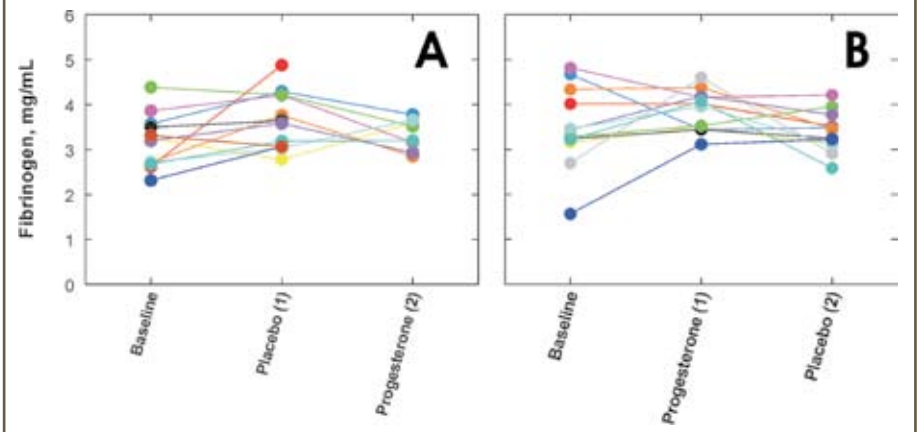

FIGURE 5. Fibrinogen levels in study individuals. Group 1 is shown in panel A and group 2 in panel B.

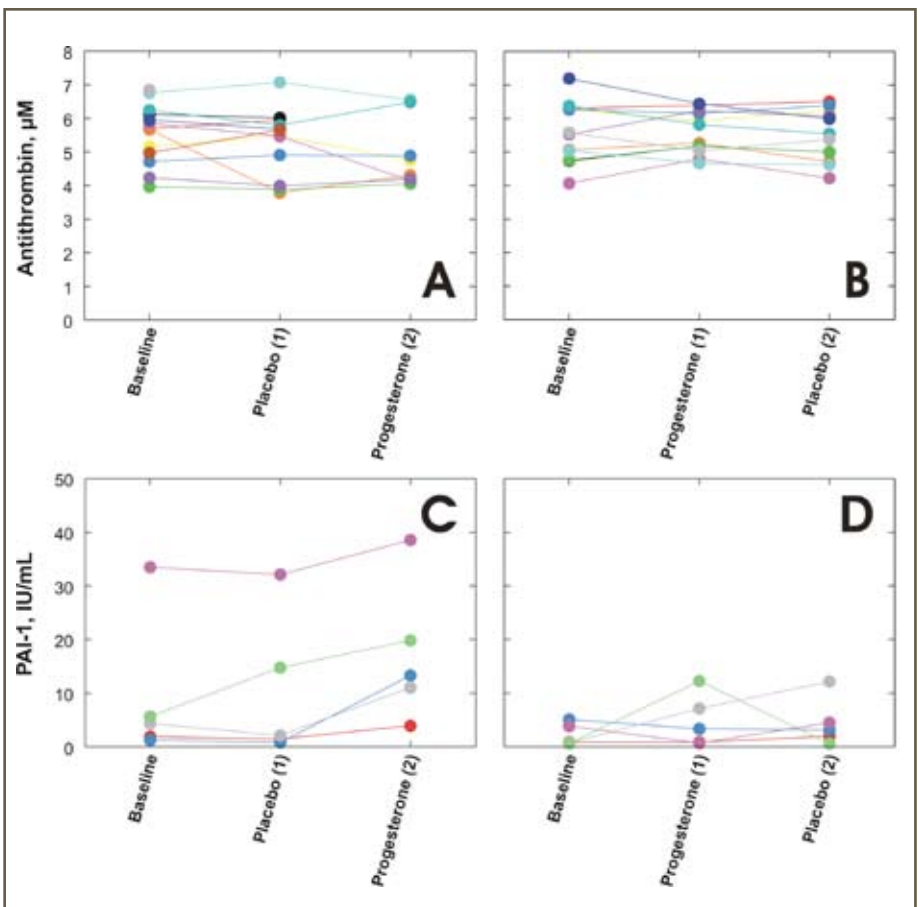

FIGURE 6. Active antithrombin III levels (top panels) and PAI-1 levels (bottom panels) in study individuals. Panels A and C are group 1 individuals and panels B and D are group 2 individuals.

fective therapy for relief of menopausal symptoms.

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Disclaimer

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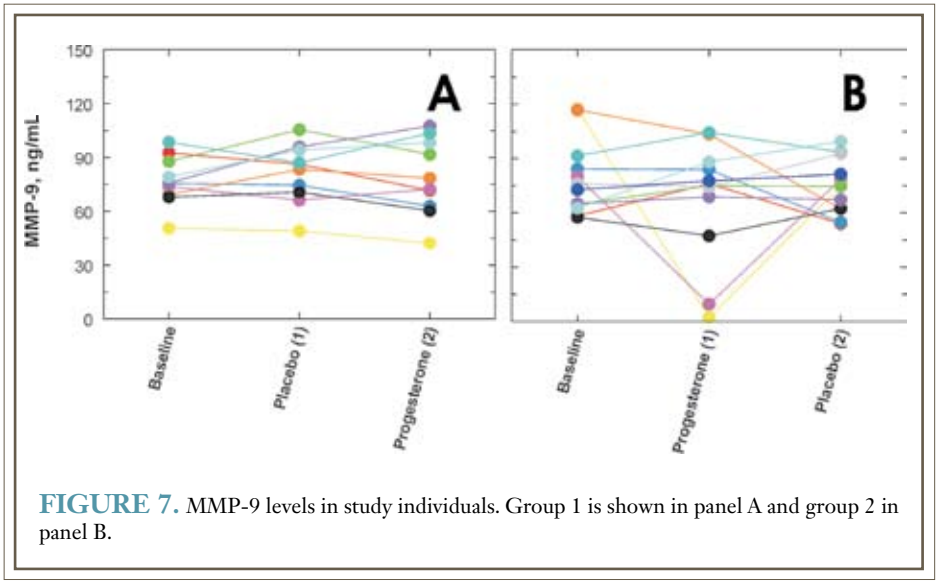

FIGURE 7. MMP-9 levels in study individuals. Group 1 is shown in panel A and group 2 in panel B.

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Address correspondence to Kenna Stephenson, MD, FAAFP, Department of Family Medicine, The University of Texas Health Science Center, 11937 US Highway 217 N, Tyler, TX 75708. E-mail: kenna.stephenson@uthct.edu