High endogenous estradiol is associated with increased venous distensibility and clinical evidence of varicose veins in menopausal women

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Objective: The purpose of this study was to determine if there is an association between elevated sex hormones (ie, serum estradiol, sex hormone binding globulin [SHBG], testosterone) and increased venous distension and clinical evidence of varicose veins in menopausal women.
Methods: Participants were 104 healthy volunteer menopausal women, aged 48 to 65 years, who were not undergoing hormonal treatment. Of these 104, 14 were excluded from analyses because their estradiol levels were compatible with a premenopausal condition (4), because they had missing values for insulin concentration (5), and because they did not show up at venous vessel examination (5). Patients underwent a physical examination to determine the presence of varicose veins; a venous strain-gauge plethysmographic examination to compute instrumental measures of venous distensibility; and laboratory analyses of blood so serum testosterone, estradiol, SHBG, glucose, and insulin could be measured. There were also prevalence ratios and odds ratios used to test the presence of an association between biochemical and instrumental variables.
Results: Serum levels of estradiol in the upper tertile of the frequency distribution were significantly associated with clinical evidence of varicose veins (prevalence odds ratios 3.6; 95% CI 1.1-11.6) and with increased lower limb venous distensibility (prevalence odds ratios 4.4; 95% CI 1.2-15.5). No association was found for SHBG and testosterone.
Conclusions: Our finding that high serum levels of estradiol are associated with clinical evidence of varicose veins and instrumental measurements indicating increased venous distensibility in menopausal women suggests that endogenous estrogens may play a role in the development of this very common venous vessel abnormalities. (J Vasc Surg 2000;32:544-9.)

Disease of the venous system of the lower limbs is a major problem affecting Western societies, resulting in considerable morbidity in the population and cost to the health service.[1] One of the factors leading to primary venous dysfunction is an abnormal venous wall distensibility.[2] Very high estradiol levels during pregnancy are associated with the clinical appearance of varicose veins.[3] High levels of estradiol in nonpregnant women could lead to varicose veins because of increased vein distensibility. Data on the association between endogenous sex hormones and the presence of varicose veins or instrumental evidence of increased venous distensibility of the lower limbs are not available in nonpregnant women.

Varicose veins are considered a risk factor for venous thromboembolism by British and European guidelines.[4,5] However, the evidence for a major role played by endogenous estrogens, and in particular estradiol, in affecting venous thromboembolism is still lacking in the literature.

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Competition of interest: nil.
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0741-5214/2000/$12.00 + 0 24/1/107768
doi:10.1067/mva.2000.107768

In this study we have evaluated the relationship between sex hormone pattern (ie, serum estradiol, sex hormone binding globulin [SHBG], testosterone) and clinical evidence of varicose veins and plethysmographic measurement of venous distensibility in a group of healthy menopausal women to provide information on a potential promoting condition leading to venous dysfunction in the lower limbs.

METHODS

Subjects. Using newspaper and broadcasting advertisement, we invited healthy female volunteers, aged 48 to 65 years, to participate in the DIANA Project (a randomized controlled trial on the effect of some nutritional changes on sex hormone pattern) in autumn 1995 through winter 1996 from the Milan area, Northern Italy. The DIANA (DIet and ANdrogens) Project is a nutritional trial; the objective is to test the effect of a specific diet on the levels of serum testosterone with other effects on sex and metabolic hormones. The measurements analyzed in this article were taken before the trial started. Eligibility criteria for a woman's participation in the DIANA Project were

1. menopause for at least 2 years;
2. no history of bilateral ovariectomy;
3. no hormonal replacement treatment for at least 6 months;
4. no history of cancer or vascular disease (in particular, no previous venous thromboembolism);
5. no adherence to vegetarian or macrobiotic diet, or to any other diet prescribed for medical reasons;
6. no treatment for diabetes;
7. agreement to participate after signing an informed consent.

Because one of the main objectives of this project was to reduce testosterone levels through a nutritional approach, 104 of 312 women recruited were selected because of their serum testosterone level (included in the upper tertile) to participate in the trial. Written informed consent to participate in a study for research purposes was obtained from all women before enrollment. The Scientific and Ethical Committee of the National Cancer Institute, Milan, Italy, approved the research with human volunteers.

Design. Baseline observation of the women who were recruited included clinical diagnosis of varicose veins, a venous strain-gauge plethysmographic measurement of venous distensibility, anthropometric measurements (weight and height), and blood samples collection.

Laboratory analyses. Blood samples were collected from the patients between 9:00 and 10:30 AM after an overnight fast. Serum for hormonal assays was stored at –30°C for a short time and then at –80°C. Circulating hormones were measured with the use of commercial kits: radioimmunoassay kits were purchased from Orion Diagnostic (Turku, Finland) for testosterone and estradiol (tailored for postmenopausal condition); immunoradiometric assay kits were purchased from Farmos (Oulunsalo, Finland) for SHBG; and microparticle enzyme immunoassay kits were purchased from Abbott (Abbott Park, Ill) for insulin. The coefficients of intra-assay and interassay variations of eight replicates for each hormone analyzed were 4.2% and 12.5% for a testosterone value of 0.420 ng/mL; 5.2% and 11.1% for an estradiol value of 10 pg/mL; 3.5% and 6.7% for an SHBG value of 34.0 nmol/L; and 2.5% and 4.6% for an insulin value of 14.2 μIU/mL. For insulin, the serum was analyzed within 2 weeks since collection.

Clinical examination of legs. Patients were examined while standing after a delay of 3 minutes. Two medical research fellows (trained in internal medicine with a qualified experience in angiology) examined the legs of the women who participated in the study. They classified the varicose veins as trunk varices (dilated trunks of the long or short saphenous veins or their principal branches), reticular varices (dilated or tortuous superficial veins that did not belong to the main trunk or its major branches), and hyphenweb varices (intradermal varices). The clinical classification of the women recruited into the study, according to the CEAP classification,[6] was 52 C_0 (no visible or palpable signs of venous disease), 10 C_1 (telangiectases or reticular veins), 26 C_2 (varicose veins), and 2 C_3 (edema). Because only trunk varices are associated in women with leg symptoms such as heaviness or tension, aching, and itching,[7] we have considered in our data analyses only trunk varices (28 patients), and we have computed in the control group the patients with only telangiectases or reticular veins (10 patients). There were no skin changes ascribed to venous disease in the women participating in the study. Ten of 28 women with varicose veins had telangiectases or reticular veins, too.

According to the etiologic classification of chronic venous disease, all the women had primary varicose veins. The anatomic classification of venous disease in the 28 women with clinical evidence of varices was 22 had venous disease only in the superficial veins and six had venous disease in the superficial and perforating veins. According to the patho-

Table I. Characteristics of the study participants (90 women)

Age (y)*	57.5 (3.8)
BMI (kg/m^2)*	27.2 (5.0)
Estradiol (pg/mL)*	8.7 (3.7)
SHBG (nmol/L)*	39.7 (18.2)
Testosterone (ng/mL)*	0.43 (0.12)
Glucose (mg/dL)*	94.6 (22.7)
Insulin (mU/L)*	6.2 (5.4)
MVIV (mL/100 mL)*	2.0 (0.5)
Varices (y/n)	28/62

*Mean (SD).

physiologic classification of venous disease, the clinical signs and symptoms of venous dysfunction were, in all the cases, results of reflux.

Duplex scanning of the veins of the lower limbs was not performed in our population study. However, the values of maximum venous outflow, a sensitive marker of venous obstruction as determined by venous occlusion strain-gauge plethysmography, were in the normal range in all the participants to the study.

Plethysmography. After the clinical examination in the same morning, the women underwent a plethysmographic examination of their lower limb venous distensibility. The patient lay on an examination bed in a supine position in a comfortable room (20-22°C) with her legs slightly elevated to facilitate venous drainage. That was accomplished by placing the thighs on a low pillow and by supporting the feet on foam block 20 cm high. Two cuffs measuring 22 × 70 cm were wrapped around the thighs, and two tension detectors (strain-gauge), whose unstretched length are about 90% of the circumference of the limb, were fixed around the calves in the point of maximum circumference. The strain-gauge was connected to the plethysmograph that transforms the volume modifications into electric tension variations and reports these variations in a graph. During the examination, the cuffs began inflating at 50 mm Hg, and the compression was kept until a relative stable calf volume had been achieved because the capacitance vessels were filled: as much blood flows in as flows out. The incremental volume represents a measure of the quantity of blood the examined district was able to receive because of its distension and is called *venous capacitance* or *maximal venous incremental volume (MVIV)* and represents a measure of venous distensibility. It is expressed in milliliters per 100 mL volume. Limbs with varicose veins have the largest venous distensibility.[8] After that the cuffs were rapidly deflated; the downward slope was a function of emptying speed of the previous venous pooling of the leg. It is measured as the maximum venous outflow and is expressed in milliliters per 100 mL per minute.

Statistical analysis. Four women were excluded because they had serum estradiol concentrations compatible with a premenopausal condition. Five women had missing values for the fasting insulin level. Five women were not present at venous vessel examination. Therefore, final comparisons were based on 90 women. Body mass index (BMI), fasting serum glucose, and insulin were judged a priori to be potential confounding variables and were included in the model to calculate the adjusted prevalence odds ratios. We computed prevalence ratios as an approximate estimate of cumulative incidence and modeled prevalence odds ratios with logistic regression analysis with SPSS Windows 95 release 7.0 (SPSS, Inc, Chicago, Ill) as an approximation to incidence ratios.[9]

RESULTS

Descriptive features of the 90 women are reported in Table I. Of 90 women, 28 (31%) had clinical varices (C_2 + C_3), and 62 (69%) had no visible or palpable signs of venous disease or only telangiectases or reticular veins (C_0 + C_1). The average MVIV values for women with varices were 2.3 mL/100 mL; the corresponding values in the absence of varices were 1.9 mL/100 mL ($P < .001$). We tested the association between both the dichotomous variable varices (yes/no) and MVIV (upper tertile versus others) with estradiol, SHBG, and testosterone. Upper tertile versus others of estradiol (first tertile: 2.1-6.5; second tertile: 6.6-9.6; third tertile: 9.7-18.6 pg/mL), SHBG (first tertile: 14.2-30.1; second tertile: 30.3-48.1; third tertile: 48.2-122.1 nmol/L), and testosterone (first tertile: 0.161-0.368; second tertile: 0.372-0.465; third tertile: 0.467-0.857 ng/mL) were used as independent variables, with varices and MVIV as dependent variables. Adjustment for BMI and fasting glycemic and insulinemic values as covariates was performed in the model, because of their relationship with sex hormone pattern shown at the Pearson correlation matrix and of the knowledge of the key role of insulin in the regulation of plasma concentrations of SHBG and of ovarian sex steroid production. There were nonsignificant differences comparing women in the lower and in the second tertiles for each hormonal variable.

The women with serum levels of estradiol in the upper tertile of the frequency distribution had a sig-

Table II. Prevalence ratios and prevalence odds ratios (95% CI) of varices and MVIV in relation to serum estradiol, SHBG, and testosterone

	Estradiol above 9.70 (pg/mL)			*SHBG above 48.10 (nmol/L)*			*Testosterone above 0.465 (ng/mL)*		
	*Prevalence ratio**	*Prevalence odds ratio*		*Prevalence ratio*	*Prevalence odds ratio*		*Prevalence ratio*	*Prevalence odds ratio*	
		Crude	*Adjusted†*		*Crude*	*Adjusted†*		*Crude*	*Adjusted†*
Varices (y/n)	P_1N_1 = 15/28 P_0N_0 = 13/62 Ratio = 2.55 (1.4-4.6)	4.2 (1.6-11.1)	3.6 (1.1-11.6)	P_1N_1 = 7/28 P_0N_0 = 21/62 Ratio= 0.74 (0.3-1.5)	0.7 (0.2-1.8)	0.9 (0.3-2.7)	P_1N_1 = 13/32 P_0N_0 = 15/58 Ratio= 1.57 (0.9-2.9)	1.9 (0.7-4.8)	1.9 (0.7-4.9)
MVIV above 2.1 (mL/100-mL)	P_1N_1 = 12/28 P_0N_0 = 15/62 Ratio = 1.77 (1.0-3.3)	2.5 (1.0-6.5)	4.4 (1.2-15.5)	P_1N_1 = 9/28 P_0N_0 = 18/62 Ratio = 1.11 (0.6-2.1)	1.1 (0.4-3.0)	1.2 (0.4-3.5)	P_1N_1 = 9/32 P_0N_0 = 18/58 Ratio = 0.91 (0.5-1.8)	0.9 (0.3-2.4)	1.0 (0.4-2.7)

*Prevalence ratio: proportion of women with varicose veins in the exposed group (P_1N_1) divided by the proportion in the reference group (P_0N_0); prevalence ratio = $(P_1N_1)/(P_0N_0)$.
†Adjusted for BMI, glucose, and insulin.

nificant association with each dependent variable tested as shown by the adjusted prevalence odds ratios comparing the upper tertile of hormonal values with the others (Table II). Prevalence odds ratio for the association of estradiol with varices was 3.6 (95% CI, 1.1-11.6); for MVIV it was 4.4 (95% CI, 1.2 -15.5). Actually, 15 (54%) of the 28 women with varices were above the upper tertile of estradiol distribution, whereas only 13 (21%) of 62 women without varices were in that range. No association has been found for SHBG and testosterone (Table II). Prevalence odds ratio for the association of SHBG in the upper tertile with varices was 0.9 (95% CI, 0.3-2.7); for MVIV it was 1.2 (95% CI, 0.4-3.5). Prevalence odds ratio for the association of testosterone in the upper tertile with varices was 1.9 (95% CI, 0.7-4.9); for MVIV it was 1.0 (95% CI, 0.4-2.7).

DISCUSSION

To the best of our knowledge, this is the first article in which the association between measured endogenous sex hormones and varicose veins plus venous distensibility in menopausal women is examined. Indirect information on the relationship between endogenous estrogens and varicose veins derives from observation in pregnancy. In earlier articles, investigators had observed that pregnancy, which implies exposure to very high estradiol levels, increased the clinical appearance of varicose veins (the production of estradiol causes the relaxation of smooth muscle and softening of collagen fibers) and their regression immediately after child delivery.[3,10] It was long thought that the gravid uterus could be responsible for the appearance of varicosity by compressing the iliac vessels and inferior vena cava[11]; however, varicose veins appear in the first trimester before the size of the uterus is large enough to compress the pelvic vessels. Moreover, a great fibromatous uterus does not cause varicose veins.[12]

The prevalence of varicose veins varies among different populations. Its prevalence is very low in African or Australian aborigine populations (0%-5%)[13,14] and is very high in Western countries (25%-75%).[15-19] Different epidemiologic terminology, population sampling, and varicose vein definition account for much of the variation in the literature. In an Italian survey the prevalence of varicose veins in the elderly population of the Campania region (1319 subjects; mean age, 74 years) was 35.2% in women. Interestingly, a high percentage of women (38.2%) reported menopause as a time starting point of varicose veins.[20]

We report a prevalence of varicose veins in 31% of menopausal women. Studies on hormone replacement therapy use and venous thromboembolism do not provide data on the effect of hormone replacement therapy on direct measurement of venous distensibility or presence of varicose veins in meno-

pausal women. The lack of this information is probably due to the attitude of physicians to consider the presence of varices a counterindication for the use of hormonal therapy.

In our data, age was not associated with peripheral vein abnormalities (increased venous distensibility and varicose veins) because the study participants were selected in a small age range. Although they were selected for testosterone levels, they had the classic characteristics of a large number of menopausal women, such as high prevalence of overweight and lower limb varicosity.

We found that the higher the serum estradiol levels were, the higher the prevalence of varicose veins and the plethysmographic measures of increased venous distensibility was. According to our results, at an estradiol value greater than 9.7 pg/mL, there is a significant excess risk of poor peripheral venous wall condition in menopause. The results are found both for clinically diagnosed varicose veins and plethysmographic measures of venous wall distensibility. The overlap observed in our study (21% of women without varicosities have high estradiol levels) implies that other factors should play a role in determining the appearance of varices of the lower limbs.

Both BMI and insulin were correlated with the presence of varices in our data; serum estradiol levels correlated strongly and positively with BMI and also with insulin serum levels. In menopausal women, the synthesis of estrogens depends primarily on the extragonadal aromatization of androgens into estrogens in adipose tissue. Moreover, a large body of evidence[21-24] suggests a key role for insulin in the regulation of SHBG plasma concentrations[25] and possibly also in the regulation of ovarian sex steroid production.[22,26,27] The observed correlation between SHBG and insulin is in line with this current theory. The association between higher estradiol levels and varicosity or increased venous distensibility still remains after adjustment for BMI and insulin. Although it is not easy to draw conclusions on causality, in our study it is likely that the residual estradiol level may play a role in influencing venous distensibility and varices. Our study is confined to those women who have a high level of serum testosterone. One cannot exclude different results at different levels of serum testosterone; however no direct or indirect evidence on the potential influence of serum testosterone is available in the literature. We did not measure progesterone and cannot draw conclusions on the interaction between progesterone and estradiol; however, it should be said that no data in the literature support the association between venous function impairment and progesterone in menopausal women not taking hormonal replacement therapy.

Our original results suggest that measurement of serum estradiol may be of importance when a hormonal therapy is planned in a woman entering menopause. This measurement may be especially beneficial to those women with serum estrogens comparable with those detectable in a perimenopausal condition (about 10-50 pg/mL). In fact, hormonal replacement therapy use could result in higher estrogenic activity since hormonal replacement therapy does not suppress residual estradiol ovarian production. In a recent article it was demonstrated that hormonal therapy increases arterial compliance in postmenopausal women.[28] It should be noted that in our sample, estradiol levels ranging between 9.7 and 18.6 pg/mL (third tertile) are significantly associated with varicose veins and increased venous distensibility.

This finding is of clinical interest and matches most of the clinical concerns about the use of hormonal therapy in early menopause without taking individual characteristics into account. This had been expressed for the potential risk of cancer because of hormonal replacement therapy,[29,30] but it is reasonable also for the potential venous disease risk.[31-34]

Thanks to the Association “Attivecomeprima” for the hospitality to the study.

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Submitted May 12, 1999; accepted Mar 24, 2000.