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Elevated Serum Estradiol/Testosterone Ratio in Men With Primary Varicose Veins Compared With a Healthy Control Group

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The role of sex hormones in men with varicose veins remains unclear. Therefore, we set up a prospective pilot-study. In 34 men, venous blood was sampled during morning hours, for the determination of serum estradiol (E2), dehydroepiandrostendion, androstendion, and free testosterone (fT). Serum E2:fT ratio was calculated. The study protocol also included patient history, physical examination, color duplex ultrasound of both limbs, and assignment of CEAP clinical stage (C) classification. About 21 symptomatic varicose men (VM [$C \geq 2$] mean age of 40.3/$\pm$6.9 years) and 13 healthy men (HM [$C \leq 1$] mean age of 38.1/$\pm$ 7.4 years) were analyzed. The serum E2:fT ratio (VM 2.83/$\pm$ 0.79 and HM 2.32/$\pm$0.63) was significantly different ($P < .05$) between the two groups. No major differences were seen on the serum levels of the sex hormones. In summary, our results demonstrate a changed serum E2:fT ratio among men with varicose veins compared to healthy men. By the fact of a small study sample, the interpretabillity of this result is limited.

Keywords: varicose veins; sex hormones in men; chronic venous disease

Introduction

Varicose veins are common in humans. The Bonn Vein Study made by the German Society of Phlebology reported a frequency of every sixth man and every fifth woman to have chronic venous insufficiency.[1] Risk factors such as age, familial transmission, obesity, and standing posture at work have been described to promote varicosity.[2,3] In women, pregnancies and high serum levels of estradiol are associated with clinical evidence of varicose veins. No association was found for sex hormone binding globulin (SHBG) or testosterone.[4,5] In men, there are also some information that hormones play a role in the development of varicose veins. Howell et al reported that men with chronic venous disease are less fertile, more adipose, and have a bigger body length.[6] Patients with Klinefelter's syndrome (characterized by the karyotype 47,XXY) have hypogonadism with an androgen deficiency and relative high estrogen levels. In these patients, the prevalence of varicose veins, which are often complicated with leg ulceration, is greater than in normal men.[7] So it is possible that beside a relative high estrogen level, also an androgen deficiency promote the building of varicose veins. The role of sex hormones (serum estradiol, dehydroepiandrostendion [DHEA-S], androstendione, free testosterone) in the association between men with varicose veins and healthy men remains unclear. Therefore, wet set up a prospective pilot-study to analyze this association.

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Material and Methods

A total of 34 men (21 varicose men [VM], 13 healthy men [HM]) entered the study. Patients (VM) were recruited from the files of our clinic. Of the 33 men between 24 and 50 years approached, 21 participated and 12 were excluded because of refusal of the informed consent. The control group (HM) was recruited from the staff in our hospital. The first 13 healthy volunteers (men between 20 and 50 years) who agreed to participate formed the control group. All participants gave their informed written consent for inclusion in the study. During each participant's visit, a standard set of information was collected (age, body mass index [BMI], family history of chronic venous disease, short medical history, and medication intake). Then we assessed all limbs for the presence of varicose veins and complete clinical severity, etiology or cause, anatomy, pathophysiology (CEAP) classification.[8] A duplex ultrasound scanning (transducer frequencies between 5 and 7.5 MHz) included measuring characteristics of outflow, and reflux was performed for the lower limb. Venous flow was examined in longitudinal sections using color flow. Pulsed Doppler was used for measuring the reverse flow. Femoral, midthigh femoral, above-knee popliteal, and distal posterior tibial veins were examined for deep venous reflux. The great saphenous vein (GSV) and the small savenous vein (SSV) were imaged continuously from the respective femoral or popliteal junction to the paramalleolar level. Reflux was defined as reverse flow longer than 0.5 seconds in the superficial veins or 1.0 seconds in the deep veins, following a manual compression-release maneuver. The examination was done with the patient standing. All symptomatic legs underwent a surgical removal of varicose veins. Inclusion and exclusion criteria are shown in Table 1.

Because of diurnal variation of serum sex hormones, we obtained blood samples from the antecubital vein only between 7.00 and 9.00 AM in 5 mL vacutainer tubes. The samples were stored for a maximum of 8 h at 2–8°C before centrifugation and separation of serum. Serum was stored at −20°C.

Dehydroepiandrostendion and Estradiol-E2 were measured by the electrochemiluminescence assays (Elecsys 2010, Roche Diagnostics, Germany). Both tests are fully automated competition immunoassays and were done according to the manufacturer's instructions for use. In brief, for the DHEA-S test, 15 μL serum were first incubated with a specific biotinylated antibody, and then with streptavidin-coated microparticles and a DHES-S derivative, labeled with a ruthemium complex. Chemilumescence of the ruthemium complex measured by a photomultiplier is indirectly proportional to the concentration of DHEA-S in the serum. The detection limit for DHEA-S in the serum is 0.1 μg/dL. For the Estradiol-E2 assay, 35 μL serum, an estradiol-specific antibody, and ruthenium-labeled estradiol peptide were used. The analytical sensitivity of the test for Estradiol-E2 is 5.0 pg/mL. Intraassay and interassay coefficients of variation were 1.7% and 4.7% for DHEA-S and 1.6% and 6.2% for Estradiol-E2, respectively. Androstendion was measured by a competetive radioimmuno assay (DSL Inc., Tex) according to the manufacturer's instructions for use. In brief, 50 μL of the serum were incubated with a specific rabbit antihuman androstendion polyclonal antiserum together with ^{125}I-labeled androstendione. The androstendione in the serum compete for the fixed numbers of antigen-binding sites of the polyclonal antiserum. The amount of bound ^{125}I-labelled androstendione and, therefore, of measured radioactivity is inversely proportional to the concentration of androstendione in the serum. The detection limit of the assay is 0.02 ng/mL. Intraassay and interassay coefficients of variation were 2.7% and 7.0% for androstendione. Free testosterone was also measured by a competitive radioimmuno assay (DPC, Los Angeles, Calif) according to the manufacturer's instructions for use. In brief, 50 μL of the serum were incubated in a tube coated with a specific monoclonal antihuman testosterone antibody together with ^{125}I-labeled testosterone analog. The amount of measured radioactivity in the tube after washing is inversely proportional to the testosterone in the serum. The detection limit of the assay is 0.15 pg/mL. The interassay coefficients of variation were between 8.0% and 18.3% for different concentration ranges of free testosterone.

As testosterone and estradiol have opposed effects, an analysis of serum estradiol:free testosterone ratio (E2:fT ratio) could be more useful than individual hormone values. Therefore, we calculated the E2:fT ratio to evaluate minimal changes of estradiol or free testosterone in both groups.

Statistical Analysis

First, we performed correlation analysis using the Spearman rank correlation coefficient and a test of

Table 1. Selection Criteria

	Varicose Men	Healthy Men
Inclusion	Symptomatic varicose veins "C" CEAP ≥ 2	No symptomatic veins "C" CEAP ≤ 1
	Duplex sonography with reflux in superficial veins > 0.5 seconds	Duplex sonography with reflux in superficial veins < 0.5 seconds
	Reflux in the deep veins < 1.0 seconds	Reflux in the deep veins < 1.0 seconds
	Inpatient for vein surgery	
	Age: 20-50 years	Age: 20-50 years
	Informed consent	Informed consent
	Short medical history	Short medical history
Exclusion	Hormone intake	Hormone intake
	Cancer	Cancer
	Elevated liver enzyme	Elevated liver enzyme
	Acute phlepitis	Varicose veins

rho = 0 to determine whether age or BMI affected the concentrations of serum estradiol, androstendion, DHEA-S, testosterone, and estradiol/testosterone quotient. Then the Wilcoxon-Mann-Whitney *U* test was applied to compare values between the varicose and the healthy men group. *P* values < .05 were considered significant.

Results

The mean age of the patients with symptomatic venous disease (n = 21) was 40.3 years (24-50 years) with a mean BMI of 26 kg/m^2 (22-38 kg/m^2). About 13 probands recruited contemporaneously formed the healthy control group. The mean age of the healthy men group was 38.1 years (27-50 years) with a mean BMI of 25 kg/m^2 (20-38 kg/m^2). In all patients with primary varicosis, an exceeded reflux more than 0.5 s in the GSV or in the SSV vein was found. According to the "C" of CEAP, in 17 patients "C3" and in 4 patients "C4" were diagnosed. The mean duration of venous disease was 6 years (1-20 years), respectively. For treatment of the GSV and SSV incompetence high ligation and if necessary stripping of GSV or SSV was done.

Subsequently, 2 persons from the varicose patient (VM) group had arterial hypertension, other two allergic rhinitis, one had depression and one tinnitus. In the HM group, one had atopic eczema and one had pollinosis.

Serum sex hormone concentrations are reported in Table 2. In brief, the mean of free testosterone was 40.11 $\pm$ 13.32 pmol/L in the VM group and 40.27 $\pm$ 9.62 pmol/L in the HM group. Androstendion was found in the mean of 4.23 $\pm$ 1.56 nmol/L in the VM group and 4.19 $\pm$ 0.92 nmol/L in the HM group. The mean serum levels of estradiol were 110.97 $\pm$ 52.09 pmol/L in the VM group and 89.12 $\pm$ 17.02 pmol/L in the HM group. DHEA-S was found in mean of 70.19 $\pm$ 23.46 μmol/L in the VM group and 93.56/44.2 μmol/L in the HM group. There were no significant differences while comparing the above-named sex hormone concentrations between the VM and HM groups. However, the E2:fT ratio of the VM group (2.82 $\pm$ 0.79) was significantly different from the E2:fT ratio of the HM group (2.32 $\pm$ 0.63) (Table 3).

Discussion

Varicose veins of the lower limbs are a common, multifactorial disease process, characterized by symptoms produced by the venous hypertension that results from structural changes and abnormalities of the venous wall.[9] The abnormal distensibility of the venous wall, proposed to be at the origin of the disease, may result from genetic factors, facilitated by environmental influences such as prolonged hydrostatic pressure and hormonal influences.[10,11] Especially, sex hormones may contribute to this phenomenon. In the present pilot study, we compared the concentrations of the sex hormones (serum estradiol, DHEA-S, androstendion, free testosterone, and estradiol/testosterone ratio) in men with varicose with that in age-matched healthy control persons.

Our results demonstrate a changed serum E2:fT ratio among men with varicose veins compared to healthy men. To the best of our knowledge, this is the first article in which this phenomenon is examined.

Information on the relationship between endogenous estrogens and varicose veins derives from observation in women. Ciardullo et al[12] found that

Table 2. Baseline Characteristics of Varicose Men (n = 21) and Healthy Men (n = 13)

	Varicose Men (Mean / SD)	Healthy Men (Mean / SD)
General characteristics		
Age (years)	40.29 / 6.91	38.15 / 7.44
BMI (kg/m^2)	26.54 / 3.77	25.20 / 4.30
Serum sex hormone concentrations		
Free testosterone (pmol/L)	40.11 / 13.32	40.27 / 9.62
Androstendione (nmol/L)	4.23 / 1.56	4.19 / 0.92
Estradiol-2 (pmol/L)	110.97 / 52.09	89.12 / 17.02
DHEA-S (μmol/L)	70.19 / 23.46	93.56 / 44.20
Estradiol-2 / free testosterone ratio (pmol/L)	2.83 / 0.79	2.32 / 0.63
Hypertension (n)	2	0
Allergy (n)	2	2
Current smoking (n)	4	2
Depression (n)	1	0

NOTES: BMI = body mass index; DHEA-S = dehydroepiandrostendion.

high serum levels of estradiol are associated with clinical evidence of varicose veins with increased venous distensibility in menopausal women, aged 48 to 65 years. Estrogen and androgen receptors are found in normal and in varicose veins.[13,14] Estrogens are an essential part of endocrine health in men.[15] In men, estrogen is mainly synthesized by local tissue aromatization of androgenic precursors from the testes and adrenal gland. A possible mechanism of the changed ratio could be an increasing aromatization of testosterone into estradiol at the cellular level already in young men with varicose veins. Indeed, physiologically, in elderly men, an increased aromatization of testosterone with a decline of androgen levels is a result of increasing aromatase acitivity with age and the age-associated increase in fat mass.[16]

In target tissues, sex hormones exert their effects through receptor proteins located in the nuclear compartment of the cells.[17] Estrogens can modulate vascular tone.[18] It regulates the vasoconstrictor endothelin (ET-1), which acts on stimulation of c-*fos* and c-*myc*. Importantly, estradiol can decrease the activity of ET-1.[19]

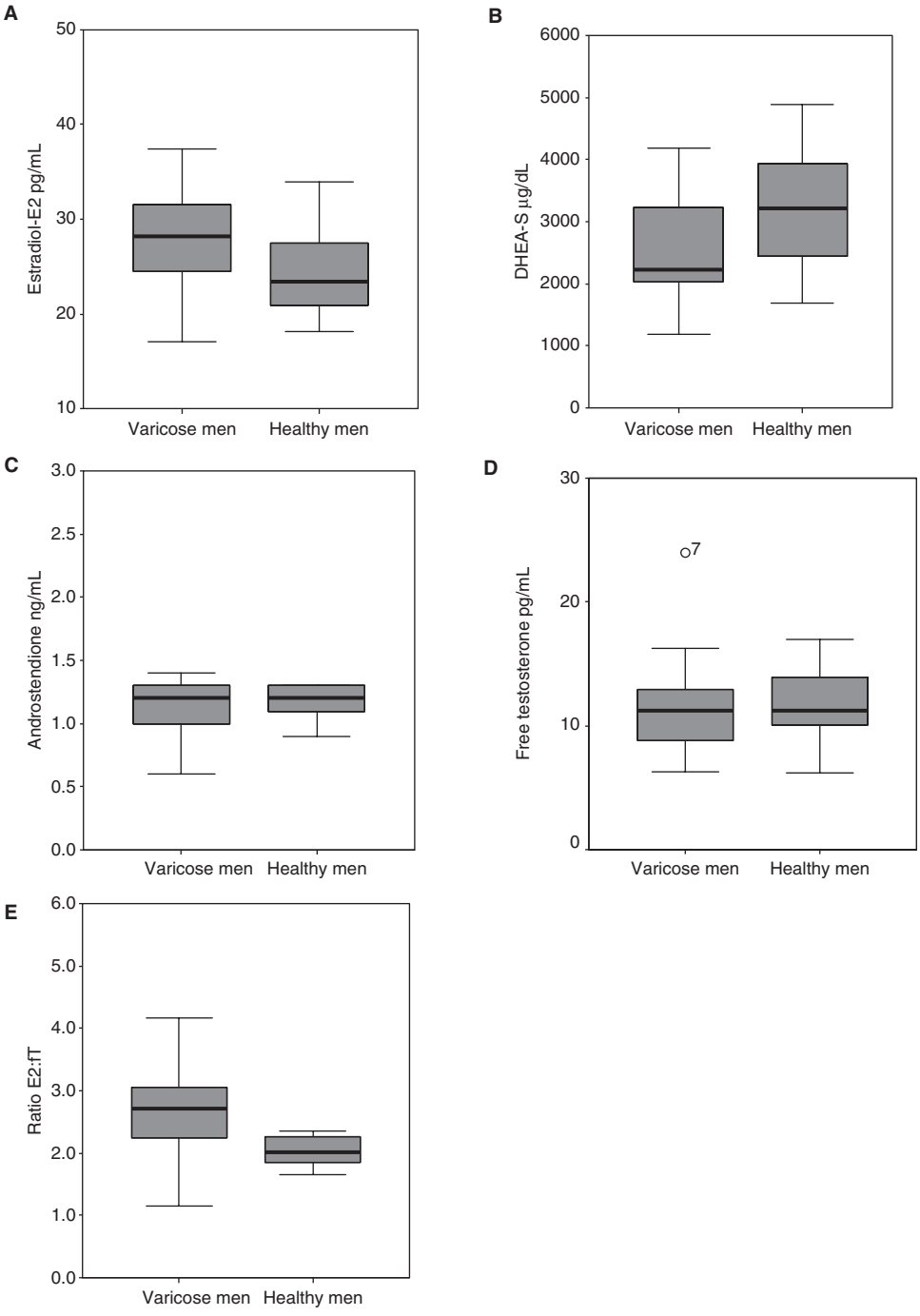
The serum androgen levels of the VM and HM groups were within the normal range, according to the present study. A new finding is a changed E2:fT ratio indicating elevated estradiol and/or decreased testosterone levels of free testosterone in the varicosity group. Androgen receptors were found in female patients with varicose veins[20]and in fibroblast,[21] an important structure in the vein wall. A modulation of enzymatic activities in the vein wall may be mode of action of androgens. In the pathogenesis of chronic venous disease, where structural proteins such as elastin and collagen are decreased, a higher activity in extracellular matrix remodeling proteins, such as the matrix metalproteases and serine proteases are reported.[22] Androgens such as dehydroepiandrosterone can degrade extracelluar matrix protein and increase procollagen synthesis.[23]

Although men do not experience an abrupt reduction in endogenous sex hormone production, it is well established that several important sex hormone levels undergo a gradual shift in men after the age of 40.[24] Therefore, men between 20 and 50 years were included in this survey and the E2:fT ratio was calculated. However, the ratio of estrogens and testosterones is held steady in age.[25]

Changes in endogenous sex hormone levels in men have an impact on cardiovascular diseases, for example, lower levels of free testosterone are associated with atherosclerosis and coronary artery disease.[26,27] This condition has been named partial androgen deficiency in aging men (PADAM) and consists in a gradual decline in sex hormone levels over years resulting in physical and psychological changes as depression, impotence, decreased sex drive, loss of muscle tone or strength, and lethargy.[28] However, the association of the endocrinologic changes and varicose veins in young men remains largely unknown.

However, various limitations of our study merit comment. The control group was formed from healthy volunteers of the hospital staff. This might be a source of bias compared to patients without varicose veins. Though few chronic diseases (hypertension, allergy, and depression) were found in our sample, conditions that may cause an alteration of sex hormone levels like liver diseases, testicular

Table 3. Sex hormones. A, Serum estradiol. B, Dehydroepiandrostendione DHEA-S. C, Androstendione. D, Free testosterone, fT. E, Ratio of E2/fT. Each box represents the median and quartiles; the low and high horizontal bars represent the extreme values. Ratio of E2/fT, $P < .05$ compared to the healthy men group.



neoplasies, or hormonal intake were excluded. Furthermore, sex hormones were measured at a single examination. However, single measures of sex hormone levels provide a reasonable estimate of long-term levels.[29]

In summary, our results demonstrate a changed serum E2:fT ratio among men with varicose veins compared to healthy men. The interpretability of these results may be limited by several factors such as a small study sample, and by the complex

interrelations of the sex hormones with other hormone systems, with common chronic diseases of aging such as cardiovascular disease, diabetes, depression, and hyperlipidemia, and with associated conditions and behavior such as obesity or sedentariness.[30] In our study, few chronic diseases (hypertension, allergy, or depression) were found and the BMI of both invested groups were in the normal range. Furthermore, blood work was limited by cost; therefore, larger studies are clearly required to consolidate our findings. It is also difficult to indicate the clinical importance of our findings. Relations between varicose veins and sex hormone levels in men, also in men with advancing age, may be of increasing importance and interest.

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