Elevated sex steroid hormones in great saphenous veins in men

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Introduction: **High serum levels of estradiol are associated with clinical evidence of varicose veins in women; however, the relationship between serum sex steroid hormones and varicose veins in men is unclear. To address this issue, serum levels of testosterone, estradiol, and androstenedione were determined in the great saphenous (GSV) and cubital veins of men with varicose veins. Messenger RNA (mRNA) expression of sex steroid hormones, metabolizing enzymes, and their receptors was investigated in tissue samples of leg veins.**

Methods: **This prospective study included 40 men, comprising 20 with varicose veins and reflux of the GSV (VM) and 20 with healthy veins (HM). All limbs were assessed by duplex ultrasound scanning of selected superficial and deep leg veins. Blood samples were taken from the cubital vein and from the GSV. Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) analysis for sex steroid hormones, their metabolizing enzymes, and receptors in saphenous veins was performed in tissue samples of varicose (n = 6) and healthy veins (n = 6).**

Results: **The VM group had significantly higher ($P < .001$) mean levels for serum testosterone (44.9 nmol/L; range, 8.8-225.1) and estradiol (242.2 pmol/L; range, 79-941) in varicose saphenous veins compared with cubital veins (testosterone, 15.5 nmol/L; range, 8.4-23.3; estradiol, 93.2 pmol/L; range, 31-147). Moreover, significantly ($P < .001$) higher mean serum estradiol levels (133.2 pmol/L; range, 63-239) were detected in the saphenous veins of the HM group compared with cubital veins (88.15 pmol/L; range, 37-153). Both groups had similar blood counts and serum androstenedione levels in the upper and lower extremity. Interestingly, qRT-PCR revealed that the mRNA expression of 5α-reductase type 1, 5α-reductase type 2, 17, 20 lyase, 17β-hydroxysteroid dehydrogenase (17β-HSD), aromatase and 3β-HSD type 2, androgen and estrogen receptor 1 was down-regulated ($P < .05$) in all samples of varicose veins vs veins obtained from healthy men.**

Conclusion: **Elevated serum estradiol and testosterone levels were detected in men with varicose veins and reflux in the GSV compared with the patient's own arm veins. Enzymes and hormonal receptors involved in steroid metabolism were down-regulated in patients with GSV reflux and varicose veins, suggestive of a negative feedback regulation. These data support the notion of a possible causal relationship between sex steroids and varicose veins in men. (J Vasc Surg 2010;51: 639-47.)**

Primary varicose veins are a common disease of the lower extremity.[1] It affects about 10% to 40% of 30- to 70-year-old individuals in industrialized countries.[2] Every sixth man and every fifth woman have chronic venous insufficiency.[3,4] In some patients with varicose veins, serious complications will develop, such as superficial vein thrombosis, dermatoliposclerosis, or venous leg ulcer. Chronic venous disease is also associated with a reduced quality of life. Predisposing factors such as familial transmission, age, obesity, and prolonged standing could have an effect in the development of varicosity.[5,6]

Although sex steroid hormones are known to influence endothelial cell function and blood viscosity, their influence and that of their receptors in the pathogenesis of varicose disease are not fully understood. In women, an association between pregnancies and high serum levels of estradiol with the clinical manifestation of varicose veins has been suggested. No association was found for sex hormone-binding globulin or testosterone.[7,8]

Several lines of evidence also support a role for sex steroid hormones in the pathogenesis of varicose veins in men. An elevated serum estradiol/testosterone ratio was found in men with primary varicose veins compared with healthy controls.[9] Howell et al[10] reported that men with chronic venous disease are more adipose, less fertile, and have an increased body height.

In patients with pelvic congestion syndrome, estrogen levels in the leg were higher than that in the arm in the presence of reflux.[11] This may point to a possible role of locally elevated estrogen levels in the varicose leg with reflux compared with the healthy arm. However, the situ-

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Competition of interest: none.

A part of the investigation data was accepted for presentation at the XVI World Meeting of the Union Internationale de Phlébologie, Fiftieth Anniversary, Monaco, Aug 31-Sept 4, 2009.

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The editors and reviewers of this article have no relevant financial relationships to disclose per the JVS policy that requires reviewers to decline review of any manuscript for which they may have a competition of interest.

0741-5214/$36.00

doi:10.1016/j.jvs.2009.07.128

ation of different serum hormone levels in arm and leg veins in men with reflux of great saphenous veins (GSVs) and in men without reflux is unclear. Therefore, we tested the hypothesis that serum testosterone and estradiol levels may be different in the leg with GSV reflux and in the healthy arm as well as in leg veins of men without reflux of GSV compared with the arm veins. For this purpose, serum and blood count measurements from GSV (with and without reflux) and brachial veins were performed. In addition, we investigated messenger RNA (mRNA) levels of sex steroid hormones, their metabolizing enzymes, and receptors in saphenous vein samples by quantitative reverse-transcription polymerase chain reaction (qRT-PCR).

MATERIALS AND METHODS

The study protocol was approved by the Ethics Committee of the University of Leipzig (AZ 049-2008). All patients provided written informed consent before undergoing any study-related procedure. The clinical study was performed as an open, prospective, single-center study at the Department of Dermatology, Venerology and Allergology at Leipzig University Medical Center, Leipzig, Germany. Surplus segments of healthy saphenous veins isolated for coronary artery bypass grafting were provided by the Department of Cardiac Surgery.

Study population. From January 2008 to September 2008, patients who were referred to our institution for surgical treatment of varicose veins, for skin changes, or healthy volunteers, were considered for inclusion. Of the 45 patients approached, 20 participated. We contacted 29 individuals for the control group, and 20 participated. A total of 40 men, comprising 20 men with reflux >500 ms of the GSV (VM) and 20 men with a competent GSV (HM) were included. Inclusion and exclusion criteria are detailed in Table I.

Table I. Selection criteria

Criteria	*Men with varicose veins*	*Healthy men*
Inclusion	Symptomatic varicose veins	No symptomatic veins
	CEAP C ≥2	CEAP C ≤1
	DUS with reflux in superficial veins >0.5 sec	DUS with reflux in superficial veins <0.5 sec
	Reflux in the deep veins, < 1.0 sec	Reflux in the deep veins, < 1.0 sec
	Inpatient for vein surgery or endoluminal treatment	
	Age: 20-80 years	Age: 20-80 years
	Informed consent	Informed consent
	Short medical history	Short medical history
	Blood withdrawal (arm/leg)	Blood withdrawal (arm/leg)
Exclusion	Hormone intake	Hormone intake
	Cancer	Cancer
	Elevated liver enzymes	Elevated liver enzymes
	Superficial thrombophlebitis	Varicose veins

DUS, Duplex ultrasound imaging.

A standard set of clinical information was collected, including age, weight, height, family history of chronic venous disease, short medical history, and systemic medication intake. We used standard equipment to measure height (m) and weight (kg) and calculated body mass index (BMI) as kg/m^2.

All limbs were assessed for the presence of varicose veins and complete CEAP classification was performed. Duplex ultrasound scanning with transducer frequencies between 5 and 7.5 MHz (Titan, SonoSite, Bothell, Wash), including measuring characteristics of outflow and reflux for the lower limb, was performed by the same experienced investigator in all patients. Venous flow was examined in longitudinal sections using color flow, and pulsed Doppler ultrasound imaging 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 GSV and the small saphenous vein were imaged continuously from the respective femoral or popliteal junction to the paramalleolar level. Reflux was defined as reverse flow >0.5 seconds in the superficial veins or 1.0 second in the deep veins after a manual compression-release maneuver. The examination was performed with the patient standing.[12] All patients with symptomatic varicose veins underwent surgical or endoluminal therapy.

Venous sample collection. Samples were obtained from six patients (age range, 55.8 ± 5.9 years; BMI, 28.2 ± 4.9 kg/m^2) undergoing surgical removal of varicose saphenous veins. Healthy segments of saphenous veins (control) were isolated from six men (age range, 64.0 ± 4.9 years; BMI, 28.5 ± 3.2 kg/m^2) undergoing coronary artery bypass grafting. The control group did not undergo duplex ultrasound scanning of the GSV before surgery. The GSV was harvested below the knee at a location from 5 to 15 cm in all cases. All veins were immediately cryopreserved in Jung tissue freezing medium (Leica Microsystems Nussloch GmbH, Nussloch, Germany) at −80°C until analysis was performed.

Serum and blood count measurements. Nonfasting blood samples were obtained in 5-mL Vacutainer tubes (BD Diagnostics, Franklin Lakes, NJ) from the antecubital vein or by ultrasound-guided puncture of a part of the GSV of the lower extremity with the individual sitting. All blood samples were obtained in the morning between 8:00 AM and 12:00 PM. The samples were stored for a maximum of 8 hours at 2° to 8°C before centrifugation and separation of serum. Serum was stored at −20°C.

Levels of testosterone and estradiol were determined by the Elecsys 2010 fully automated immunoassay system (Roche, Basel, Switzerland). Levels of androstenedione were measured by an immunoradiometric assay from DSL (Sinsheim, Germany). All other blood parameters were determined by the Modular Analyzer (Roche).

RNA preparation and complimentary DNA synthesis. Total RNA from the venous tissue was prepared using TRIZOL (Invitrogen, Germany). Briefly, the tissue (about

Table II. Primer sequences

Gene name	*Enzyme*	*Primer sequence*	*Product size (bp)*	*Temp (°C)*
17, 20 LYASE	17, 20 lyase	F: CGATCAGAAGCTGGAGAAGAT R: CACGAAGACAGGAAAGGAGAT	106	79
3β-HSD-2	3β-Hydroxysteroid dehydrogenase type 2	F: GGACCAAGCTGACTGTACTTG R: TGATGGACTCTCTGTGAGTGAC	135	80
5AR1	5α-reductase type 1	F: TCATGGAGTGGTGTGGCTA R: TTCCGGAGGTACCACTCAT	121	80
5AR2	5α-reductase type 2	F: ATTCTCAGAGGCACTGCCT R: AGAAGACACCCAAGCTAAACC	121	79
17β-HSD	17β-Hydroxysteroid dehydrogenase	F: GGCTTGTTTGAGGTTGGAG R: GCTGGCATTCTCAAAGTCAC	149	79
AROMATASE	Aromatase	F: GGACTTTGCCACTGAGTTGA R: ACAGACATGGTGTCAGGAGCT	111	79
ESR-1	Estrogen receptor 1	F: ATCATCTCGGTTCCGCAT R: TGGTCCTTCTCTTCCAGAGACT	129	78
ESR-2	Estrogen receptor 2	F: TCGTGTGAAGGATGTAAGGC R: CACTTCGTAACACTTCCGAAGT	144	77
AR	Androgen receptor	F: TTCTACCAGCTCACCAAGCT R: GGCACTTGCACAGAGATGAT	146	81
GHR	Growth hormone receptor	F: TCACCAAGCTGCCCATAT R: TGGCAGAGTGAGACCATTTC	175	80
HKG-RSP	Ribosomal protein S26	F: GCAGCAGTCAGGGACATTTCTG R: TTCACATACAGCTTGGGAAGCA	68	79

HKG, Housekeeping gene.

4-mm vein length) was thawed in 1 mL of TRIZOL and homogenized twice using an Ultra-Turrax (IKA, Nesher, Israel) homogenizer for 20 seconds each. After separation of organic and anorganic phases, the RNA was precipitated from the aqueous phase by the addition of isopropanol, as suggested by the manufacturer. The quantity and quality of the obtained RNA was determined by spectrophotometry (ND-1000, NanoDrop Technologies Inc, Wilmington, Del). Total RNA (1 μg) from each sample was used for first-strand complimentary DNA (cDNA) synthesis using M-MLV Reverse Transcriptase and Oligo(dT) (Promega, Madison, Wis), following the manufacturer's instructions.

Quantitative RT-PCR analysis. Analysis of gene expression of sex steroid hormones, their metabolizing enzymes, and receptors was done using the SYBR Green PCR Master Mix (Applied Biosystems, Darmstadt, Germany) according to the manufacturer's instructions. The primers used were designed using Primer Express 2.0 software (Applied Biosystems, Table II). The reaction mixtures were amplified for 40 cycles at an annealing temperature of 55°C using an ABI PRISM 7000 instrument (Applied Biosystems). The assessed data were evaluated using the Sequence Detection 1.2.3 software (Applied Biosystems). Ribosomal protein S26 was used as an endogenous control for the relative quantification of the target messages. The primer concentration optimization and the absence of nonspecific products were confirmed by performing dissociation curve analysis, which resulted in single products at specific melting temperatures.

Statistical analysis. Results were analyzed using descriptive statistics and are presented as mean ± standard deviation. Correlation analysis (Spearman rank correlation coefficient and a test of $\rho = 0$) was performed to determine whether height or weight affected the concentrations of serum sex steroid hormones or blood count. Nonparametric statistics (median and quartiles; Wilcoxon-Mann-Whitney) were used. Statistical significance for the RT-PCR results was calculated by the two-sample independent-groups *t* test. The statistical significance was defined by values of $P < .05$. All calculations were performed using the SPSS 11.5 software (SPSS Inc, Chicago, Ill) and Statistica 7.1 software (Statsoft Software, Tulsa, Okla).

RESULTS

Patient demographics, clinical parameters, laboratory data parameters, and "C" of CEAP classification are reported in Table III and in part in Fig 1. Further supporting the findings of the study is that the VM group was significantly older than the HM group.

In brief, the mean serum levels of estradiol in the VM-arm and VM-leg groups demonstrated a significant difference (arm/leg difference, $P < .001$, Table III, Fig 1, *1a*). Also in the HM group, estradiol levels in the legs and arms were significantly different (arm/leg difference, $P < .001$; Table III, Fig 1, *2a*). A similar observation for the estradiol level was made for the C_0 subgroup (n = 12; arm/leg difference, $P = .002$) and C_1 subgroup (n = 8; arm/leg difference, $P = .012$). Moreover, mean of testosterone levels in the VM-arm and in the VM-leg groups displayed a significant difference (arm/leg difference, $P < .001$; Table III, Fig 1, *1b*), whereas no differences could be demonstrated in HM (Table III, Fig 1, *2b*). Interestingly, the mean serum levels of estradiol and testosterone in the VM-leg and HM-leg groups demonstrated a significant difference (estradiol, $P = .021$; testosterone, $P =$

Table III. Baseline characteristics of men with varicose veins and healthy men

Variable	*VM (n = 20) Mean ± SD or No.*	*HM (n = 20) Mean ± SD or No.*
General characteristics		
Age, y	57.3 ± 10.8	45.7 ± 16.9
Body mass index, kg/m^2	27.0 ± 3.2	25.2 ± 3.0
Serum sex hormone levels and blood count		
Estradiol, pmol/L (40-161)[a]		
Arm	93.2 ± 28.5 (93.0)[b]	88.2 ± 32.7 (99.0)[b]
Leg	242.2 ± 208.6 (189.5)[b]	133.2 ± 42.9 (125.5)[b]
Testosterone, nmol/L (9.9-27.8)[a]		
Arm	15.5 ± 5.0 (16.3)[b]	16.1 ± 6.0 (15.0)[b]
Leg	44.9 ± 61.1 (25.0)[b]	18.0 ± 10.0 (14.6)[b]
Androstenedione, nmol/L (1.04-10.7)[a]		
Arm	5.1 ± 1.9	4.6 ± 1.7
Leg	7.3 ± 5.0	4.8 ± 1.8
Hemoglobin, nmol/L (7.8-10.8)[a]		
Arm	9.3 ± 0.6	9.2 ± 0.5
Leg	9.4 ± 0.7	9.2 ± 0.6
Leucocytes, 10^9/L (4.0-9.0)[a]		
Arm	6.0 ± 1.4	6.5 ± 1.7
Leg	6.1 ± 1.4	6.5 ± 1.8
Platelets, 10^9/L (150-300)[a]		
Arm	243.2 ± 1.4	237.4 ± 40.9
Leg	246.8 ± 47.2	238.5 ± 44.2
Comorbidities		
Hypertension	8	5
Allergy	1	1
Psoriasis	0	1
Hypercholesterolemia	1	0
COPD	1	0
Current smoking	6	4
Medication		
Antihypertensive drugs	8	5
NSAIDs	2	3
Sympathomimetic	1	0
Allopurinol	1	0
TNF inhibitor	0	1
CEAP C		
C_0	0	12
C_1	0	8
C_2	2	0
C_3	9	0
C_4	7	0
C_5	1	0
C_6	1	0

COPD, Chronic obstructive pulmonary disease; *HM,* healthy men; *NSAIDs,* nonsteroidal anti-inflammatory drugs; *VM,* varicose veins.
[a]Normal reference range.
[b]Median value.

.04; Fig 1, *3a,b*). No significant differences were found when arm and leg levels of androstenedione, hemoglobin, leucocytes, and platelets were investigated in VM or HM (Table III, Fig 1, *1c*).

To address a possible confounding of the association between serum sex steroid hormones or blood count by height or weight and age, these parameters were analyzed. In the VM group, there was no significant association between age and serum sex steroid hormones or blood count. In the HM group, a significant negative correlation was found between estradiol arm and age ($r = -0.666$), estradiol leg and age ($r = -0.667$), androstenedione arm and age ($r = -0.762$), androstenedione leg and age ($r = -0.620$), hemoglobin arm and age ($r = -0.662$), and hemoglobin leg and age ($r = -0.679$). In contrast, a significant positive correlation in the VM group was found between hemoglobin arm and weight ($r = 0.443$), hemoglobin leg and weight ($r = 0.510$), leucocytes leg and weight ($r = 0.446$), leucocytes arm and height ($r = 0.382$), and platelets leg and height ($r = 0.401$). A significant negative correlation was found between estradiol arm and height ($r = -0.643$). Finally, a positive correlation between estradiol arm and height ($r = 0.634$) and estradiol leg and height ($r = 0.508$) was observed in the HM group. A negative correlation existed between testosterone arm and weight ($r =$

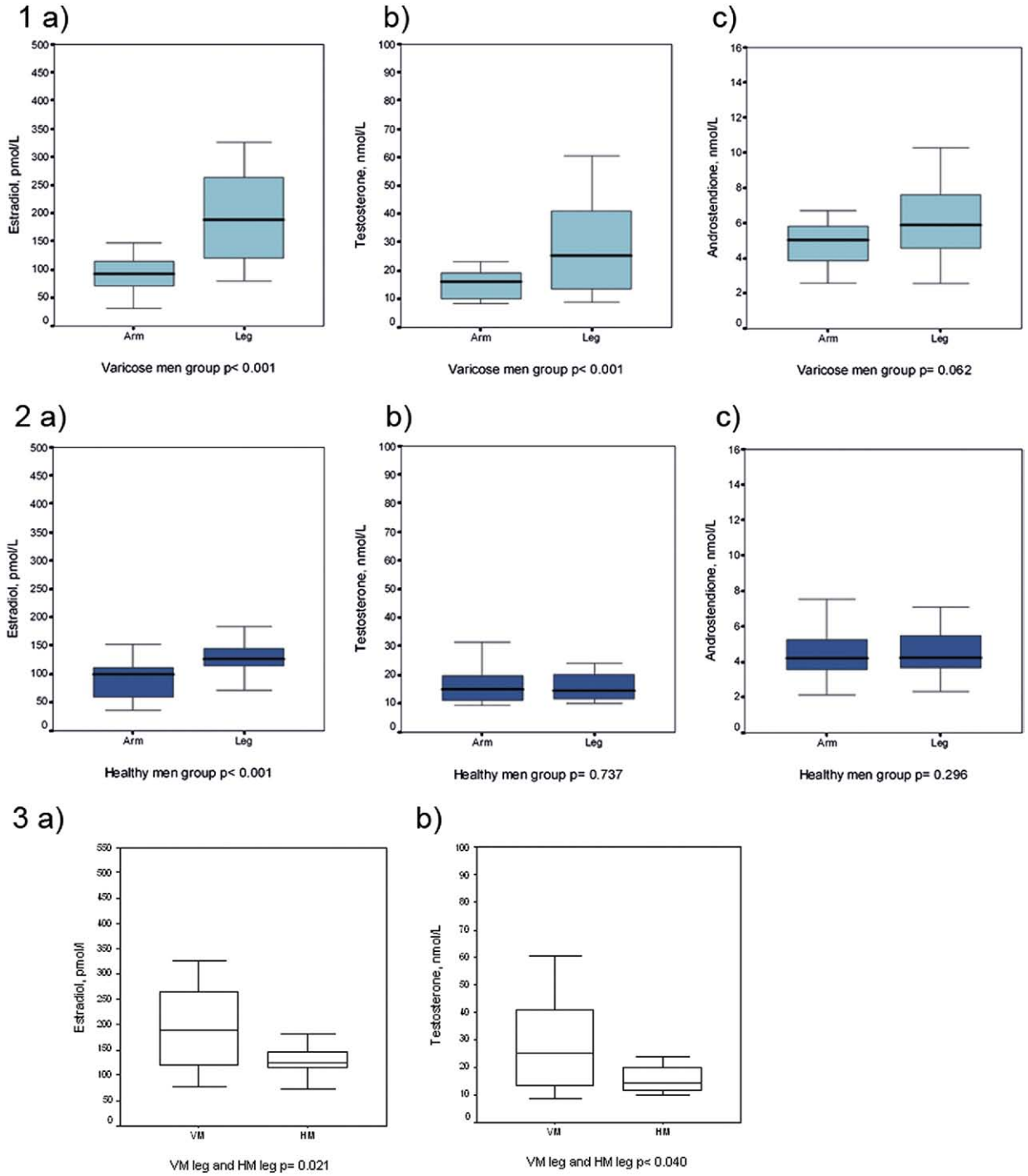

Fig 1. Serum level of **(a)** estradiol, **(b)** testosterone, and **(c)** androstenedione in the arm/leg of **(1)** men with varicose veins *(VM)* and **(2)** healthy men *(HM)*. **3,** Serum level of estradiol and testosterone in legs of VM and HM. The edge of each box represents the quartiles, the horizontal line designates the median, and the whiskers represent the extreme values (outliners not shown).

−0.438), testosterone leg and weight ($r = -0.419$), and hemoglobin leg and height ($r = -0.568$).

Quantitative RT-PCR analysis revealed that the gene expression of 17, 20 lyase, 5α-reductase type 1, 5α-reductase type 2, 17β-hydroxysteroid dehydrogenase (HSD), aromatase, and 3β-HSD type 2 was significantly downregulated in varicose vein samples from patients compared with controls (Fig 2, *a-f*). Further, the gene ex-

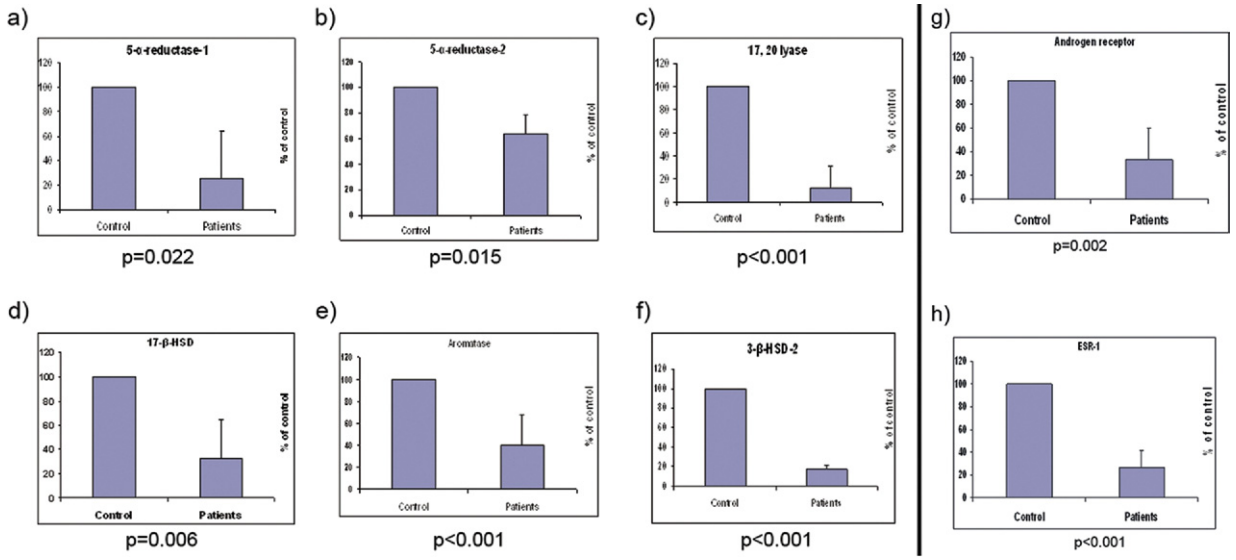

Fig 2. Enzymes involved in steroid metabolism were down-regulated in samples from patients compared with controls: **(a)** 5α-reductase type 1, **(b)** 5α-reductase type 2, **(c)** 17, 20 lyase, **(d)** 17β-hydroxysteroid dehydrogenase *(HSD)*, **(e)** aromatase, and **(f)** 3β-HSD type 2 expression. Gene expression of the enzymes of control samples was set at 100%, and messenger RNA (mRNA) gene expression of the enzymes in patient samples was calculated as the percentage of the change from control. Hormone receptors involved in steroid metabolism were down-regulated in patient samples compared with control. Expression of **(g)** androgen receptor and **(h)** estrogen receptor (ESR) type 1 was down-regulated in patient samples compared with controls. Gene expression of the hormone receptors of control samples was set at 100%, and mRNA gene expression of the enzymes in patient samples was calculated as the percentage of the change from control.

pression of estrogen receptor 1 and androgen receptor was significantly down-regulated in patients' samples compared with controls (Fig 2, *g, h*). On the other hand, mRNA levels for estrogen receptor 2 were undetectable in samples from patients and controls.

DISCUSSION

This pilot study documented that serum estradiol levels of the GSV part of the lower extremity were significantly higher than those of the antecubital vein of the upper extremity in men with GSV reflux >500 ms with varicose veins (VM) as well as in healthy male controls (HM). Interestingly, only in the presence of reflux (VM) are serum levels of testosterone in GSV significantly higher than in the arm. No difference between leg and arm was seen in androstenedione levels and blood count parameters in the VM and HM groups. Regarding the mean age of the VM and HM groups, however, the VM patients were significantly older than the HM group. The decline with age in serum levels of hormones in men is known as *partial androgen deficiency* in aging men.[13] Our study also showed a discrete decline in the HM group of the serum level of testosterone with age in the arm and leg blood sample. Interestingly, in the VM group, the arm and leg serum level of testosterone remained age-independent (arm) or increased (leg), but the role of this finding remains to be determined.

The process of varicose vein formation is probably multifactorial and a complex process.[14] An abnormal distensibility of the venous wall has been proposed as the origin of the disease. Varicose veins can be formed in any segment of the limb. In particular, tributaries that connect to axial veins can be varicose without any reflux in the communicating vein. Risk factors found to be associated with varicose veins include genetic factors facilitated by environmental influences such as prolonged hydrostatic pressure, pregnancy, age, greater height, and obesity.[15,16] The occurrence of venous stasis during pregnancy and the presence of sex steroid hormone receptors in saphenous veins have suggested a sex hormone dependency of the venous pathology.[17] Sex hormones may contribute to this phenomenon, because high serum levels of estradiol are associated with increased venous distensibility and varicose veins in menopausal women.[4] In these studies, however, blood was always taken from the arm veins under the assumption that the level of sex hormones should be similar in all peripheral vessels.

The importance of hormones and their metabolism for the cardiovascular system in men has been elucidated quite recently.[18,19] Sex hormone receptors are found in both varicose and healthy GSVs.[20-22] Interestingly, low levels of estrogen receptors were found in the varicose part of the saphenous veins,[23] which is in line with our own data presented here. In target tissues, sex hormones exert their effects through receptor proteins located in the nuclear compartment of the cells[24] and through cell membrane action.[25] Steroids have been shown to elicit cellular re-

sponses in a rapid manner even when prevented from entering the cell. In particular, a rapid rise of intracellular calcium concentration is observed in a variety of cell types.[26] Expression of androgen and estrogen receptor type 1 in saphenous veins was demonstrated in the present study. Interestingly, the expression of both receptors was lower in GSVs with reflux than in healthy ones. This is likely to be of relevance for vascular effects of estrogen/testosterone or susceptibility to disease, or both. However, the low level of androgen and estrogen receptor type 1 and the absence of estrogen receptor type 2 in most GSVs with reflux could be either specific to the saphenous vein or due to a loss of the receptors during the degenerative process at the presence of GSV reflux and symptomatic varicose veins. Then again, GSVs used for coronary artery bypass grafting have also some degree of pre-existing fibrotic changes in the wall.[27]

The vascular endothelium has a crucial role in the regulation of vascular homeostasis by controlling coagulation, inflammatory responses, and vascular tone. Estradiol may influence the vascular reactivity[28] due to increased nitric oxide (NO) expression and increase NO production and subsequent vasorelaxation. Estrogen also has the capacity to suppress the endothelial adhesion of monocytes.[29] Further, oral estrogen increases the risk of venous thromboembolism in postmenopausal women.[30] Whether the physiologic role of estradiol is similar in veins and arteries and whether it is similar in men and women remains unknown.

The serum level of testosterone was significantly different between the veins in the arm compared with the level in the leg in presence of varicose veins with reflux in the GSV. The normal range of testosterone is 9.9 to 27.8 nmol/L. In the VM group with a GSV reflux >500 ms, the level of testosterone showed a mean of 44.85 nmol/L (range, 8.82-225.1 nmol/L). Testosterone, the main androgen in the circulation, is mainly protein-bound either to the sex hormone-binding globulin or to albumin.[31] We could exclude, however, that differences in sex hormone-binding globulin serum levels account for the differences in testosterone levels between arm and leg in the VM group (data not shown).

At present, we can only speculate on the mechanisms responsible for the differences in sex hormone levels in the venous blood of arms and legs. In men, the predominant sites of secretion for androgens are the interstitial Leydig cells of the testis[32] and, only to a smaller extent, the adrenal glands. Aromatase, an enzymatic complex localized in the endoplasmic reticulum of numerous tissues, ensures the conversion of androgens into estrogens.[33] One possible explanation for the observed differences could be a reflux from gonadal veins or adrenal veins, especially with standing position, into the main saphenous veins. Perhaps tributary veins could be investigated in formalin-fixed bodies. At the moment, however, we cannot provide any evidence. On the other hand, sex steroids, in addition to their gonadal source, can also be synthesized by peripheral tissues.[34] We thus tested whether a synthesis of sex steroid hormones can also occur in GSVs with reflux. Our data show that at least at the mRNA level, the saphenous vein expresses key enzymes involved in steroid synthesis. Interestingly, these enzymes are down-regulated in GSVs with reflux and varicose veins compared with the healthy GSVs, a finding that suggests a negative feedback regulation. Indeed, high source levels may lead to reduced expression of the metabolizing enzymes to avoid the production of high levels of active metabolites. As a consequence, venous blood flow may be directly modulated by steroid hormones.

Limitations of the present study include the small study sample with only white men, which may not be representative of all men who develop a reflux of the GSV and varicose veins. Especially in the VM group, only two were from C_2 and the rest were from more advanced C classes, so that reflux of GSV with varicose veins may not be the only thing in the comparison. For further investigations, patients should be selected in by C grouping, such as only C_2, C_3 or only C_5, C_6 and compared with a control group without any signs of venous reflux or signs of chronic venous disorders.

The interpretability of these results may be limited by the complex interrelations of the sex hormones with other hormone systems, with common chronic diseases of aging such as cardiovascular disease, diabetes, hyperlipidemia, and with associated conditions and behavior such as obesity.[35]

CONCLUSIONS

The vasculature is controlled by a plethora of growth and inhibitory modulators;[36] therefore, our findings may simply be an epiphenomenon in such a complex system. On the other hand, our observation of different serum concentrations of important sex steroid hormones (estradiol and testosterone) in patients with GSV reflux and symptomatic varicose veins and healthy arm veins may have implications for the pathogenesis of varicose veins in men. Certainly, the regulation of sex steroid serum levels in the varicose and healthy segments of peripheral veins desires further clinical and laboratory studies.

Authors MK, EM participated equally and share first authorship. Authors CZ and JS share senior authorship.

We are indebted to all patients who participated in this study and to the staff of the Department of Cardiac Surgery, Heart Center, University of Leipzig for their support. We thank Bettina Hentschel, Institute of Medical Informatics, Statistics and Epidemiology, Leipzig University Center, for her valuable assistance in supervising the study design and helping analyzing the data.

AUTHOR CONTRIBUTIONS

Conception and design: MK, EM, TW
Analysis and interpretation: MK, EM, CZ
Data collection: MK, EM, JG, UA
Writing the article: MK, EM
Critical revision of the article: MK, EM, JG, TW, CZ, UA, JS
Final approval of the article: MK, JG, CZ, JS

Statistical analysis: MK, EM, JG
Obtained funding: JS, CZ
Overall responsibility: MK

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Submitted May 20, 2009; accepted Jul 18, 2009.

INVITED COMMENTARY

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Sexual steroid hormones are known to influence the endothelial function of blood vessels. Several studies have shown an association between increased levels of estradiol and varicose veins in women. Also, the effect of pregnancy in the diameter of the veins and the development of reflux and varicose veins has been demonstrated. Until the current study, few reports suggested a possible association between sexual steroid hormones and varicose veins in males. The important finding in this report is that estradiol and testosterone serum levels were higher in refluxing veins of the lower extremities when compared to those from the arm veins of the same patients. It is also interesting that the serum levels of estradiol and testosterone were significantly higher in lower ex-