# Heritability of Venous Function in Humans

Marina Brinsuk, Jens Tank, Friedrich C. Luft, Andreas Busjahn, Jens Jordan

*Objectives*—Venous function contributes to the pathogenesis of thrombophlebitis, venous thrombosis, and possibly to arterial hypertension. Venous disease is presumably heritable; however, the genetic variance of venous function is unknown.

*Methods and Results*—We determined the heritability of venous function in 46 twin pairs (24 monozygotic, age $35\pm11$ years, 14 men, 34 women; 22 dizygotic, age $30\pm8$ years, 19 men, 25 women). After a resting phase in the supine position, we determined venous function in both legs by impedance plethysmography. Venous capacity was determined by a standardized protocol. In addition, we obtained venous pressure volume curves by slowly deflating a thigh cuff from 60 to 0 mm Hg. Venous compliance was determined as the steepest part of the venous pressure volume curve. Heritability was estimated using a path modeling approach. Unadjusted heritability was 0.6 ($P<0.05$) for venous capacity and 0.9 ($P<0.05$) for venous compliance. The heritability estimate for venous capacity decreased to 0.3 after adjustment for age, body mass index, and body fat. The heritability estimate for venous compliance remained essentially unchanged after adjustment for sex and age.

*Conclusions*—We conclude that venous function is strongly influenced by genetic factors. The genes involved may influence venous disease states. (*Arterioscler Thromb Vasc Biol*. 2004;24:207-211.)

**Key Words:** twin study ■ genetics ■ plethysmography ■ venous compliance

Approximately 75% of the circulating blood volume is contained in veins. Therefore, relatively small changes in venous function may have a substantial effect on the cardiovascular system. For example, an increase in venous tone may cause a shift of blood volume from the periphery toward the heart and increase cardiac output. This mechanism may contribute to increased cardiac output in the earlier stages of essential hypertension.[1,2] Venous function becomes particularly important during assumption of upright posture as 500 to 1000 mL are pooled below the diaphragm.[3] Variability in the amount of blood that is pooled during standing influences the propensity to experience orthostatic symptoms.[4] Venous function may also contribute to the pathogenesis of varicose veins, thrombophlebitis, and venous thrombosis. Thus, genes that influence venous function might play an important role in a variety of common cardiovascular ailments. Indeed, ≈60% of the variation in the susceptibility to venous thrombosis is related to genetic factors.[5] A positive family history substantially increases the likelihood to suffer from varicose veins,[6] vasovagal syncope,[7] or essential hypertension. However, the research on the genetics of all these conditions has largely neglected the possibility that venous function may be an important intermediate phenotype. The responsiveness of veins to adrenergic stimulation is influenced by genetic factors.[8,9] Yet, the mechanical properties of veins, such as compliance and capacity, may be more relevant to physiological function. The magnitude of the genetic contribution to these measures is not known. We determined the heritability of venous function in a cohort of normal twins.

## Methods

### Subjects

We investigated the heritability of venous function in 46 twin pairs. Twenty-four twin pairs were monozygotic (MZ, age $35\pm11$ years, 14 men, 34 women) and 22 twin pairs were dizygotic (DZ, age $30\pm8$ years, 19 men, 25 women). The twin pairs were recruited from the Berlin Twin Register.[10] The subjects underwent a medical history interview and physical examination before the study. Only healthy persons were included in the study. Persons ingesting medications except birth control pills were excluded from the study. Zygosity was determined using 5 microsatellite markers co-amplified by polymerase chain reaction. The probability of a DZ twin pair to share all marker alleles by chance is 0.006. The overall rate of correct classification is >99%.[11] Written informed consent was obtained before study entry as required by the institutional review board.

### Study Protocol

Subjects were evaluated at the Franz Volhard Clinical Research Center after an overnight fast. The level of physical activity was assessed with a questionnaire.[12] Height and weight measurements were taken. Skinfold thickness was measured with a caliper (triceps, chest, abdomen, thigh, calf) to estimate body fat content. In addition, we assessed body composition with body impedance analysis (Quadscan 4000, Bodystat Ltd, Isle of Man, Great Britain). Relative body
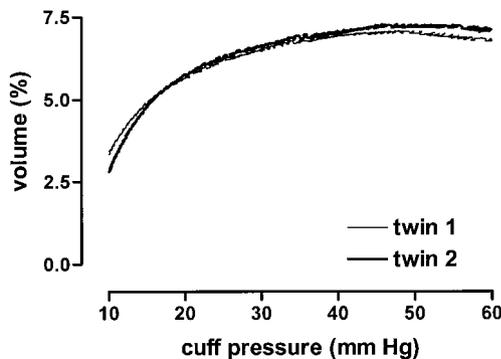
**Figure 1.** Pressure volume relationship determined by impedance plethysmography in the left leg in a monozygotic twin pair. The venous occlusion cuff was inflated to 60 mm Hg. After four minutes cuff inflation, the cuff pressure was lowered from 60 to 0 mm Hg at a rate of 1 mm Hg/sec. Venous compliance was determined with linear regression analysis at the steepest part of the curve.

fat content, relative lean body mass, and relative dry lean body mass were computed. Supine blood pressure and heart rate were determined after a resting phase of at least 15 minutes with an automated brachial blood pressure cuff (Dinamap). Routine blood samples were obtained. All studies were conducted in a quiet room at 20° C during morning hours.

## Evaluation of Venous Function

Changes in leg volume were determined in both legs with impedance plethysmography using the four electrode technique (Rheoscreen, Medis, Ilmenau, Germany).[13–15] Changes in limb volume were expressed as ml/100 mL tissue (%). These measurements tend to be independent of body size. Venous-collecting cuffs were placed around the thigh proximal to the knee. The sampling field was located at the inner side of the calf at the largest circumference. Sampling electrodes were placed proximal and distal to the largest circumference 10 cm apart from each other. Current injecting electrodes were placed on the forefoot and the thigh proximal to the occlusion cuff. Venous capacity was determined by a standardized protocol. Briefly, the leg was raised by 30° to empty the veins to obtain a decrease in capacitance below ambient venous pressure. Then, occlusion cuff pressure was raised in three steps (40, 60, and 80 mm Hg). Cuff pressure was increased automatically until venous filling leveled off. After the completion of this testing, the leg was lowered to 10° and venous pressure-volume curves were obtained.[16,17] The thigh cuff was inflated to 60 mm Hg for four minutes. This period is sufficient for almost complete venous filling.[16] Then, the cuff pressure was lowered from 60 to 0 mm Hg at a rate of 1 mm Hg/sec by an automated system. When cuff pressure is released at such a rate, intravenous pressure tracks cuff pressure.[16] Cuff pressure was plotted against leg volume using data

obtained with 10 to 60 mm Hg occlusion pressure.[16] The correlation between cuff pressure and intravenous pressure is lost at lower cuff pressures. Figure 1 shows venous pressure-volume curves in an MZ twin pair. Venous compliance was determined as the steepest part of the venous pressure volume curve using linear regression analysis. We compared the effect of venous occlusion time on venous compliance in seven subjects. Venous compliance was 0.05±0.01, 0.05±0.01, and 0.06±0.02%/mm Hg after 2, 4, and 8 minutes venous occlusion, respectively.

## Statistics and Quantitative Genetics

Statistical analysis was conducted using the SPSS program (SPSS Inc., Chicago, IL). All data are expressed as mean±SD. Relationship between parameters was assessed by linear regression analysis. Interindividual differences of mean group values were tested with unpaired *t*-test. A value for $P<0.05$ was considered to be statistically significant. Parameters of the quantitative genetic models were estimated by structural equation modeling using the MX program developed by Neale.[18] The variability of any given phenotype within a population can be decomposed into genetic influences ($Var_{addGen}$), environmental influences shared by the twins within a family ($Var_{sharedEnv}$), and effects of random environment ($Var_{env}$):

$$Var=Var_{addGen}+Var_{sharedEnv}+Var_{env}.$$

For MZ and DZ, the covariance of their phenotype is given by:

$$Cov_{MZ}=Var_{addGen}+Var_{sharedEnv};$$

$$Cov_{DZ}=0.5\ Var_{addGen}+Var_{sharedEnv}.$$

Heritability analysis in twin studies can estimate additive components of genetic variability as well as two environmental influences, shared and nonshared environmental influences. These values estimate the relative amount of the variable's influence on interindividual differences up to a sum of one. Genetic as well as environmental effects were estimated by the best fitting model as selected by the $\chi^2$ value.[19] Adjustments for age, body mass index (BMI), and blood pressure were done by multiple linear regression using unstandardized residuals. In case of significant deviations from normal distribution, the appropriate transformations were applied. To test relations between measures of venous function and potential covariates, correlations were calculated for the total sample as well as for males and females separately. As many covariates were not independent of one another, stepwise multiple regression analysis was applied to determine a minimum set of predictor variables. The setting for a stepwise exclusion of variables was placed at a probability value of >0.5.

## Results

MZ and DZ twins had similar age, body mass index, resting blood pressure, and resting heart rate (Table 1). DZ twins were more commonly male, a difference reflected in their values for body fat and lean body mass. Venous function was

**TABLE 1.    Baseline Characteristics, Intra-Class Correlation Coefficients, and Heritability**

|  | MZ | DZ | Total | r MZ | r DZ | h² |
|---|---|---|---|---|---|---|
| No. | 48 | 44 | 92 | | | |
| Male/female | 14/34 | 19/25 | 33/59 | | | |
| Age, y | 35±11 | 30±8 | 32±10 | | | |
| Body mass index, kg/m² | 23.5±4,5 | 23.6±3,2 | 23.6±3,9 | | | |
| Body fat, kg/% | 14±6/22±8* | 13±7/18±8 | 14±6/20±8 | | | |
| Lean body mass, kg/% | 50±9*/78±8 | 58±12/82±8 | 54±11/80±8 | | | |
| Venous capacity, % | 6.0±2.2 | 5.1±2.3 | 5.6±2.3 | 0.60 | −0.06 | 0.60* |
| Venous compliance, %/mm Hg | 0.24±0.14* | 0.18±0.12 | 0.21±0.13 | 0.90 | 0.25 | 0.90* |

MZ indicates monozygotic twins; DZ, dizygotic twins; h², heritability; and r, intra-class correlation coefficients. *$P<0.05$

**TABLE 2.    Correlations With Venous Function**

|  | Capacity | Compliance |
|---|---|---|
| Age | 0.17 | 0.30* |
| Body mass index | −0.16 | −0.21* |
| Waist circumference | −0.21 | −0.13 |
| Body fat content, % | 0.25* | 0.41* |
| Lean body mass, % | −0.25* | −0.41* |
| Dry lean mass, % | −0.38* | −0.37* |
| Supine SBP | −0.33* | −0.37* |
| Supine DBP | 0.12 | 0.19 |
| Supine heart rate | 0.00 | 0.02 |
| Physical activity | −0.08 | −0.23* |



**Figure 3.** Correlation in venous capacitance between siblings in monozygotic and dizygotic twins. The correlation was much stronger in monozygotic twins compared with dizygotic twins.

highly variable. For example, venous compliance ranged from 0.06 to 0.67%/mm Hg. However, venous function was similar in MZ and DZ twins when adjusted for age- and sex-related effects. We assessed the relationship between venous function and several anthropometric measures (Table 2). Body composition and systolic blood pressure were directly and inversely correlated with venous functional data, respectively. Moreover, we observed an age- and sex-related effect on venous function.

Venous compliance and venous capacity were highly correlated in MZ twins (Figures 2 and 3). The correlation was much weaker or even absent in DZ twins. For the heritability estimation, venous compliance and venous capacity were adjusted for sex and age. Adjusted venous capacity and compliance were closely related in MZ but not in DZ twins (Table 1). These findings are consistent with a strong genetic influence on venous function. The heritability estimates were 0.60 for venous capacity and 0.90 for venous compliance.

To test whether or not this genetic influence was secondary to genetic influences on body composition, we adjusted our measures of venous function. For venous capacity, the linear regression included age, BMI, and the weight of body fat as significant predictors. The heritability of the adjusted capacity was reduced to 0.3. For venous compliance, the regression included age, sex, BMI, and absolute and relative weight of body fat. The heritability after adjustment was 0.89 and thereby remained virtually unchanged.

## Discussion

Twin studies have been extensively used to characterize the interaction of genetic and environmental factors on cardio-
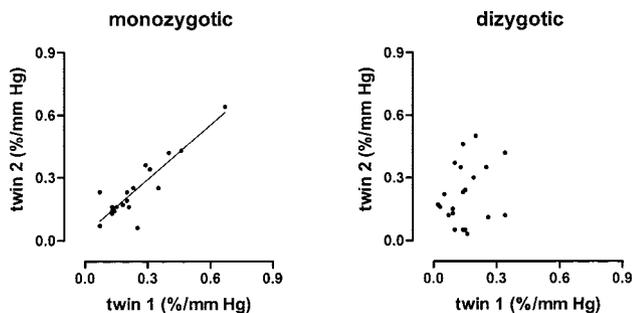


**Figure 2.** Correlation in venous compliance between siblings in monozygotic and dizygotic twins. The correlation was much stronger in monozygotic twins compared with dizygotic twins.
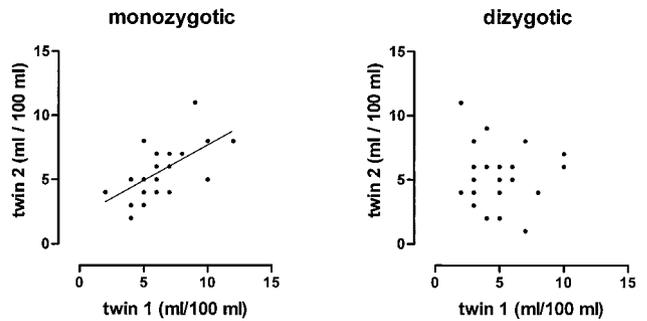
vascular phenotypes.[12,20] We employed the twin approach to test the hypothesis that venous function is influenced by genetic factors. We obtained different measures of venous function using the impedance plethysmography technique.[13,14] Our data suggest that both venous compliance and venous capacity are strongly influenced by genetic factors.

Genes that influence venous tone either through humoral or by neural mechanisms could mediate a genetic effect on venous function measurements. Indeed, genetic factors contribute to the variability of venous responsiveness to adrenergic stimulation.[8,9] Adrenergic stimulation shifts the venous pressure-volume curve to lower volumes.[16,17] The shift might influence the venous capacity measurements. However, in healthy subjects and in orthostatic intolerance (POTS, postural tachycardia syndrome) patients, the shape of the venous pressure curve and, thus the venous compliance, is essentially unchanged during sympathetic stimulation.[16,17] Therefore, it is unlikely that our heritability estimate of venous compliance is solely explained by a genetic effect on venous adrenergic tone.

We hypothesize that the strong heritability of venous function may be explained by genes that influence venous structure. Genes that influence vascular remodeling or that encode for connective tissue components are potential candidate genes for future association studies. For instance, varicose veins possess greater quantities of matrix metalloproteinase (MMP)-1 than normal veins and demonstrate regional variation in MMP-1 and MMP-13.[21] Another potential candidate is the gene encoding for collagen III, which is deficient in the saphenous veins of patients with varicose veins.[22]

The magnitude of the venous distention at a given intravascular pressure is not only related to venous structure, but also to the mechanical properties of the surrounding tissue. The mechanical properties may be modulated by a variety of factors, including tissue composition and interstitial fluid content. An effect of tissue composition on venous compliance is implicated by a recent study that showed a reduction in venous compliance in sedentary persons compared with endurance-trained subjects.[23] The heritability estimate for venous capacity was attenuated after adjustment for body composition and body mass index. In contrast, the heritability estimate for venous compliance was unchanged after statistical adjustment. Our findings suggest that the genetic influence on venous capacity, but not venous compliance, is

mediated in part by genes that modulate body composition. Because we did not obtain a measure for microvascular filtration or interstitial fluid content in the leg, we were not able to assess a potential genetic link between these measures and venous function.

The genetic effect on venous function might also be important to the pathogenesis of cardiovascular disorders, such as arterial hypertension, chronic venous disease, and orthostatic intolerance. Indeed, the rather high heritability estimates in our study suggest that venous function may be an important intermediated phenotype. Reduced venous compliance has been described in patients with essential hypertension,[2] a disorder that is highly heritable. Therefore, the genes that influence venous compliance may also be relevant for the pathogenesis of arterial hypertension. We present functional data. However, we speculate that subsequent varicosities may be the result of functional attributes that are largely inherited. Indeed, chronic venous disease is strongly influenced by genetic variance.[24,25] This issue was also addressed in an elaborate genetic analysis of 68 large pedigrees that included 80 nuclear families.[26] The authors found an astoundingly strong genetic variance, namely penetrance frequencies between 70% and 92%. Finally, abnormalities in venous compliance have been described in patients with orthostatic intolerance.[17]

Our study necessarily has some limitations. One potential weakness is that we characterized genetic influences on venous function in a cohort of healthy subjects. Nevertheless, venous disease is common and will likely occur in many of our subjects as they grow older. The genes involved may be extremely difficult to find. However, genes involved in Mendelian diseases were shown to act as quantitative trait loci in the general population, supporting the close relationship between physiological and pathological processes.[27,28] Another potential limitation is the methodology and protocol we employed to assess leg volume. Two methods are widely available to determine leg volume changes, namely impedance plethysmography, as in our study, or strain gauge plethysmography. The correlation between the two methods was found to be good in an earlier study.[15] The cuff inflation duration and the leg position, as well as the cuff pressure-profile,[29] may have confounded venous function and may have caused a systematic deviation from the "true" venous compliance and capacity. We also cannot exclude the possibility that venous filling was incomplete during our measurements, which may have caused us to underestimate the venous capacity. Another potential limitation is the fact that we used cuff pressure as a surrogate parameter for intravenous pressure to determine venous pressure-volume curves.[17] The relationship between cuff pressure and intravenous pressure may be altered in individuals with increased resting venous pressure.[30] Finally, we cannot exclude the possibility that increased microvascular filtration during occlusion cuff inflation may have led to secondary changes in venous function measurements. Indeed, the applied cuff pressures were certainly above isovolumetric venous pressure.[31] However, we did not see a major difference in the compliance measurements after 2, 4, and 8 minutes of venous occlusion. The strong heritability estimates suggest that even if our data deviated systematically from "true" venous function, they were nevertheless highly reproducible.

Despite these issues, we suggest that venous capacity and compliance are inherited and that both linkage and association studies are suitable to pursue specifically which gene loci and genes are involved. Elucidation of the genes that influence venous function may provide important new insight in the pathophysiology of cardiovascular disorders that are associated with disordered venous function.

## Acknowledgments

## References

1. Freis ED. Studies in hemodynamics and hypertension. *Hypertension.* 2001;38:1–5.
2. Safar ME, London GM, Levenson JA, Simon AC, Chau NP. Rapid dextran infusion in essential hypertension. *Hypertension.* 1979;1:615–623.
3. Smit AA, Halliwill JR, Low PA, Wieling W. Pathophysiological basis of orthostatic hypotension in autonomic failure. *J Physiol Lond.* 1999;519:1–10.
4. Streeten DH. Pathogenesis of hyperadrenergic orthostatic hypotension: evidence of disordered venous innervation exclusively in the lower limbs. *J Clin Invest.* 1990;86:1582–1588.
5. Souto JC, Almasy L, Borrell M, Blanco-Vaca F, Mateo J, Soria JM, Coll I, Felices R, Stone W, Fontcuberta J, Blangero J. Genetic susceptibility to thrombosis and its relationship to physiological risk factors: the GAIT study. Genetic Analysis of Idiopathic Thrombophilia. *Am J Hum Genet.* 2000;67:1452–1459.
6. Lee AJ, Evans CJ, Allan PL, Ruckley CV, Fowkes FG. Lifestyle factors and the risk of varicose veins: Edinburgh Vein Study. *J Clin Epidemiol.* 2003;56:171–179.
7. Mathias CJ, Deguchi K, Schatz I. Observations on recurrent syncope and presyncope in 641 patients. *Lancet.* 2001;357:348–353.
8. Gupta A, Carruthers SG. Familial studies of heritability of alpha1-adrenergic receptor responsiveness in superficial veins. *Clin Pharmacol Ther.* 1997;62:322–326.
9. Dishy V, Sofowora GG, Xie HG, Kim RB, Byrne DW, Stein CM, Wood AJ. The effect of common polymorphisms of the beta2-adrenergic receptor on agonist-mediated vascular desensitization. *N Engl J Med.* 2001;345:1030–1035.
10. Boomsma D, Busjahn A, Peltonen L. Classical twin studies and beyond. *Nat Rev Genet.* 2002;3:872–882.
11. Becker A, Busjahn A, Faulhaber HD, Bahring S, Robertson J, Schuster H, Luft FC. Twin zygosity: automated determination with microsatellites. *J Reprod Med.* 1997;42:260–266.
12. Tank J, Jordan J, Diedrich A, Stoffels M, Franke G, Faulhaber HD, Luft FC, Busjahn A. Genetic influences on baroreflex function in normal twins. *Hypertension.* 2001;37:907–910.
13. Penney BC, Narducci LM, Peura RA, Anderson FA, Wheeler HB. The impedance plethysmographic sampling field in the human calf. *IEEE Trans Biomed Eng.* 1979;26:193–198.
14. Anderson FA, Jr., Penney BC, Patwardhan NA, Wheeler HB. Impedance plethysmography: the origin of electrical impedance changes measured in the human calf. *Med Biol Eng Comput.* 1980;18:234–240.
15. Yamamoto Y, Yamamoto T, Oberg PA. Impedance plethysmography for blood flow measurements in human limbs. Part 2: influence of limb cross-sectional area. *Med Biol Eng Comput.* 1992;30:518–524.
16. Halliwill JR, Minson CT, Joyner MJ. Measurement of limb venous compliance in humans: technical considerations and physiological findings. *J Appl Physiol.* 1999;87:1555–1563.
17. Freeman R, Lirofonis V, Farquhar WB, Risk M. Limb venous compliance in patients with idiopathic orthostatic intolerance and postural tachycardia. *J Appl Physiol.* 2002;93:636–644.
18. Neale MC, Boker SM, Xie G, Maes HH. *Mx: Statistical Modeling.* 6th ed. Richmond, Va: Department of Psychiatry, 2002.

19. Neale MC, Cardon LR. *Methodology for Genetic Studies of Twins and Families*. Dordrecht, the Netherlands: Kluwer Academic Publishers, 1992.

20. Busjahn A, Voss A, Knoblauch H, Knoblauch M, Jeschke E, Wessel N, Bohlender J, McCarron J, Faulhaber HD, Schuster H, Dietz R, Luft FC. Angiotensin-converting enzyme and angiotensinogen gene polymorphisms and heart rate variability in twins. *Am J Cardiol.* 1998;81: 755–760.

21. Gillespie DL, Patel A, Fileta B, Chang A, Barnes S, Flagg A, Kidwell M, Villavicencio JL, Rich NM. Varicose veins possess greater quantities of MMP-1 than normal veins and demonstrate regional variation in MMP-1 and MMP-13. *J Surg Res.* 2002;106:233–238.

22. Sansilvestri-Morel P, Rupin A, Badier-Commander C, Fabiani JN, Verbeuren TJ. Chronic venous insufficiency: dysregulation of collagen synthesis. *Angiology.* 2003;54 Suppl 1:S13–S18.

23. Monahan KD, Dinenno FA, Seals DR, Halliwill JR. Smaller age-associated reductions in leg venous compliance in endurance exercise-trained men. *Am J Physiol Heart Circ Physiol.* 2001;281:H1267–H1273.

24. Berard A, Abenhaim L, Platt R, Kahn SR, Steinmetz O. Risk factors for the first-time development of venous ulcers of the lower limbs: the influence of heredity and physical activity. *Angiology.* 2002;53:647–657.

25. Jeanneret C, Labs KH, Aschwanden M, Bollinger A, Hoffmann U, Jager K. Physiological reflux and venous diameter change in the proximal

26. Guo Q, Guo C. Genetic analysis of varicose vein of lower extremities. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi.* 1998;15:221–223.

27. Knoblauch H, Muller-Myhsok B, Busjahn A, Ben Avi L, Bahring S, Baron H, Heath SC, Uhlmann R, Faulhaber HD, Shpitzen S, Aydin A, Reshef A, Rosenthal M, Eliav O, Muhl A, Lowe A, Schurr D, Harats D, Jeschke E, Friedlander Y, Schuster H, Luft FC, Leitersdorf E. A cholesterol-lowering gene maps to chromosome 13q. *Am J Hum Genet.* 2000; 66:157–166.

28. Busjahn A, Knoblauch H, Faulhaber HD, Böckel T, Rosenthal M, Uhlmann R, Hoehe MR, Schuster H, Luft FC. The qt interval is linked to two long-qt Syndrome loci in normal subjects. *Circulation.* 1999;99: 3161–3164.

29. Bauer A, Bruegger D, Gamble J, Christ F. Influence of different cuff inflation protocols on capillary filtration capacity in human calves: a congestion plethysmography study. *J Physiol.* 2002;543:1025–1031.

30. Stewart JM, Weldon A. Vascular perturbations in the chronic orthostatic intolerance of the postural orthostatic tachycardia syndrome. *J Appl Physiol.* 2000;89:1505–1512.

31. Gamble J, Gartside IB, Christ F. A reassessment of mercury in silastic strain gauge plethysmography for microvascular permeability assessment in man. *J Physiol.* 1993;464:407–422.

lower limb veins during a standardised Valsalva manoeuvre. *Eur J Vasc Endovasc Surg.* 1999;17:398–403.