Modifications of plasma levels of tissue factor pathway inhibitor and endothelin-1 induced by a reverse Trendelenburg position: Influence of elastic compression—Preliminary results

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Purpose: **The purpose of this study was to assess the effects of the passive 45-degree reverse Trendelenburg position and graduated compression stockings (GCS) on plasma tissue factor pathway inhibitor (TFPI) and endothelin-1 levels in a group of volunteers.**
Methods: **Ten healthy subjects lay on an examining table for 30 minutes while baseline measurements were made. The table was then tilted to a 45-degree upright position for 60 minutes, and measurements were repeated. On a different day, subjects were tilted again, but on this occasion they wore thigh-length GCS. Blood was drawn before and 60 minutes after tilting during both sessions, and plasma TFPI and endothelin-1 were obtained. Cross-sectional areas of the calf medial gastrocnemius vein, before and after tilting, were measured by a duplex scanner.**
Results: **Upright tilting induced a significant dilation of the medial gastrocnemius veins that was partially corrected by the use of elastic stockings. Similarly, endothelin-1 levels significantly increased after tilting. The use of GCS did not modify these differences. On the other hand, although TFPI levels were not affected by tilting without stockings, they were significantly elevated after tilting when GCS were used.**
Conclusions: **Upright passive tilting induces significant dilation of the deep calf veins and is associated with a significant increase in plasma levels of endothelin-1, whereas TFPI levels remain unchanged. The use of elastic stockings reduces the degree of calf distention but does not prevent an increase in endothelin-1. TFPI levels are significantly increased after tilting when GCS are used. This might represent a previously unknown mechanism of action of elastic stockings with interesting potential for deep vein thrombosis prophylaxis. More studies are warranted in a larger series to confirm these results. (J VASC SURG 1995;22:568-72.)**

Endothelial cells, which line the inner surface of blood vessels, play a key role in vascular homeostasis. The healthy vascular endothelium maintains the fluidity of blood by presenting a nonthrombogenic surface to circulating plasma proteins. On the other hand, endothelial cells also play a crucial role in the control of the tone of the blood vessel wall and thus contribute to the regulation of blood pressure. Normal endothelium responds to local changes in its environment by releasing a number of vasoactive factors that induce vessel relaxation (nitric oxide, prostacyclin) or constriction (endothelin).[1]

Tissue factor pathway inhibitor (TFPI), a recently described serine protease synthesized by endothelial cells,[2,3] is the factor Xa–dependent inhibitor of the tissue factor coagulation pathway.[4,5] TFPI is released to the blood after heparin injection.[2,6-8]

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Endothelin-1 is another recently described endothelium-derived polypeptide with potent vasoconstrictor and pressor action in most arteries, veins, and microvessels of many mammalian species.[9] Plasma endothelin-1 levels are affected by postural changes, showing significant increase by change from the supine to the upright position in healthy volunteers.[10,11]

The purpose of this study was to assess the effect of passive 45-degree reverse Trendelenburg position on plasma TFPI and endothelin-1 levels in a group of healthy volunteers and the influence of wearing elastic compression stockings on such levels.

METHODS

Ten healthy volunteers (five men and five women, mean age 30.8 years, range 20 to 42 years) were included in the study. The protocol was approved by the hospital's institutional review board, and informed consent was obtained from each subject. None of them was receiving any drugs. At 8:30 AM, after overnight fasting, subjects lay in the supine position on a tilt table. After 30 minutes, blood was drawn from an antecubital vein with a double syringe technique (baseline value), collected in citrated vacutainer tubes (Becton Dickenson, Franklin Lakes, N.J.) and centrifuged at 2000 *g* for 15 minutes at room temperature. Aliquots of plasma were immediately stored at −70° C for later use. Immediately thereafter, a baseline cross-sectional area of the right medial gastrocnemius vein was measured with a high-resolution color duplex ultrasound system (Ultramark 9 HDI; Advanced Technology Laboratories, Bothell, Wash.) as previously described.[12] All duplex examinations were performed by the same senior vascular technologist.

After completing the supine measurements, the examining table was tilted to a 45-degree reverse Trendelenburg position. Foot stoppers were not used to avoid muscle contraction of the leg that could influence the results. To prevent subjects from sliding off the examining table, they were loosely strapped by Velcro strips at the subxyphoid level. Blood samples were again collected from a different antecubital vein at 60 minutes after upright tilting, and the cross-sectional area of the calf medial gastrocnemius vein was measured once more.

Each subject was reexamined an average of 12.3 days later (range 6 to 15). The same protocol was followed with regard to duplex measurements and blood sampling. On this occasion, subjects wore fitted bilateral thigh-length graduated compression stockings (18 mm Hg at the ankle and 8 mm Hg in the thigh, TED stockings; Kendall Healthcare Products, Mansfield, Mass.).

Plasma concentration of endothelin-1 was determined by radioimmunoassay on the basis of the competition between unlabelled endothelin-1 and a fixed quantity of iodine 125–labeled endothelin-1 for a limited number of binding sites on an endothelin-1–specific antibody (Amersham, Arlington Heights, Ill.) (endothelin-1 normal range 195 to 339 pg/ml). Immunologic levels of TFPI were determined by ELISA method (American Diagnostica, Greenwich, Conn.) as described by Broze et al.[5] (TFPI normal range 60 to 105 ng/ml).

Statistical analysis consisted of calculating the median and 25% to 75% interquartile range (IQR) for the measured parameters. Wilcoxon rank sum testing was used for within-subject comparisons. The correlations between the plasma markers and vein cross-sectional areas were calculated with a linear regression model. Data were analyzed by use of the Statistical Package for Social Sciences (SPSS, Inc., Chicago, Ill.).

RESULTS

The median and IQR of the medial gastrocnemius cross-sectional areas, plasma endothelin-1, and TFPI levels are presented in Table I. There was a significant increase of the cross-sectional area of the calf vein after 60 minutes of tilting ($p = 0.007$). When stockings were used, the vein dilation was also significant ($p = 0.007$). The vein cross-sectional areas were significantly reduced when stockings were applied, both in the supine position ($p = 0.007$) and after 60 minutes tilting ($p = 0.01$).

As shown in Table I, plasma levels of TFPI did not change significantly from the supine to upright position ($p = 0.75$) when stockings were not used. However, when stockings were in use, TFPI levels increased significantly after tilting, as compared with baseline ($p = 0.005$). There was not a significant increase in TFPI levels induced by the stockings when subjects lay in the supine position ($p = 0.16$). Yet stockings were associated with significantly higher TFPI levels after tilting ($p = 0.005$).

Plasma levels of endothelin-1 increased significantly after tilting compared with the supine position both without ($p = 0.003$) and with stockings ($p = 0.02$). Neither in the supine position nor after tilting did stockings significantly modify endothelin-1 levels ($p = 0.75$ and $p = 0.5$, respectively). There were no significant correlations between the

Table I. Medial gastrocnemius vein CSA and plasma levels of TFPI and endothelin-1

	Stockings	*Before tilting*	*After tilting*	*Difference**
CSA (cm^2)	No	0.29 (0.27-0.5)	0.79 (0.6-1.1)	$p = 0.007$
	Yes	0.2 (0.12-0.36)	0.49 (0.3-0.67)	$p = 0.007$
TFPI (ng/ml)	No	63.5 (59-73.5)	68 (54-76.2)	$p = 0.75$
	Yes	71 (67.5-75.5)	83 (78.7-90)	$p = 0.005$
Endothelin-1 (pg/ml)	No	212 (174-288)	275 (240-600)	$p = 0.03$
	Yes	222 (154-279)	308 (244-406)	$p = 0.02$

CSA, Cross-sectional area.
*Wilcoxon signed rank test.
Values are expressed as median (IQR).

medial gastrocnemius vein cross-sectional areas and TFPI and endothelin-1 levels nor between TFPI and endothelin-1.

DISCUSSION

The first objective of this study was to assess the influence of passive upright tilting on plasma levels of TFPI and endothelin-1, two endothelium-related peptides that are important for the normal regulation of vascular homeostasis. The second objective was to evaluate how the use of elastic stockings would affect the changes in plasma levels of TFPI and endothelin-1 induced by upright tilting.

Our results indicate that upright tilting is associated with significant calf vein distention. In a prior study[12] we demonstrated that reverse Trendelenburg vein distention is greater in the calf veins than in the popliteal or femoral veins. Elastic compression stockings reduce venous stasis, a key thrombosis risk factor, and for this reason they are commonly used for the prevention of venous thromboembolism.[13-15] In a recent study, Coleridge-Smith et al.[16] demonstrated that compression stockings prevent venous distention in patients undergoing general surgical procedures in the supine position. In this study we have also found the same type of stockings to significantly prevent calf vein distention induced by the reverse Trendelenburg position in healthy volunteers. This is interesting because we used low-pressure stockings, and one could expect a higher degree of dilation induced by 60-minute passive upright tilting. Probably, had the tilt position been prolonged for 2 or 3 hours, the calf cross-sectional area would increase to similar values as those observed without stockings. In fact, we have previously documented a progressive calf vein dilation from 30 to 60 minutes of tilting.[12]

TFPI plays a key role in the regulation of the extrinsic coagulation pathway and is currently being investigated as a potential therapeutic agent. Several studies have shown that plasma concentration of TFPI increases twofold to fourfold after the administration of both unfractionated and low molecular weight heparin.[2,6-8,17] It has been suggested that the inhibition of factor VII activation might be as important for the antithrombotic effect of heparin as is the inhibition of factor Xa or factor IIa.[18] Moreover, some recent experimental studies have revealed antithrombotic properties of recombinant TFPI.[19,20]

TFPI plasma levels are increased after extensive physical exercise,[21] but, unlike tissue plasminogen activator, its levels are not increased by infusion of desmopressin or venous occlusion.[2,22,23] On the other hand, some studies suggest that TFPI synthesis by endothelial cells increases with shear stress.[24] Our results do not show a significant change in plasma levels of TFPI after tilting. TFPI increased significantly after 60 minutes of upright tilting when elastic stockings were used. We do not have an explanation for this surprising finding. Although the differences were nonsignificant, TFPI levels increased from 63.5 ng/ml to 68 ng/ml after tilting and in the supine position from 63.5 ng/ml to 71 ng/ml, when stockings were applied. The significant increase observed, from 63.5 ng/ml to 83 ng/ml when both stockings and tilting were present, could represent the additive result of both factors. It could be hypothesized that venous stasis induced by tilting gives rise to a release of TFPI by the endothelium to locally prevent the development of thrombosis. The addition of stockings in this setting could either modulate the TFPI release by the vein wall or facilitate its clearance into proximal veins. This could explain the higher levels detected in the systemic blood when both tilting and stockings are present. This finding needs to be validated in further studies, including larger numbers of subjects, because it could represent a previously unsuspected mechanism of action of elastic stockings with relevant potential for venous thromboembolism prophylaxis.

Endothelin-1 is released by cultured endothelial cells. Stagnation of blood flow could induce the

production of endothelin-1.[25] Furthermore, low shear stress stimulates the production of endothelin-1 by cultured endothelial cells.[26,27] Our finding of a significant increase in plasma endothelin-1 levels after upright tilting is similar to the findings of other investigators who also reported higher endothelin-1 levels with such postural change.[10,11] Although in our experience, elastic stockings significantly reduced vein distention, they did not significantly influence plasma endothelin-1 levels. This may indicate that the release of endothelin-1 after tilting is not induced by vein distention of the legs. On the contrary, plasma endothelin-1 increase after changing from the supine to the upright position could be mediated by a baroreceptor reflex, because endothelin-1 does not increase after tilting in patients with primary autonomic failure and orthostatic syncope.[11]

In conclusion, our preliminary data indicate that passive upright tilting induces a significant dilation of the calf veins that is paralleled by a significant increase of endothelin-1, whereas the plasma levels of tissue factor pathway inhibitor remain unchanged. Although elastic compression of the legs does not prevent a significant increase in endothelin-1, it reduces the degree of calf deep vein distention and significantly increases the levels of tissue factor pathway inhibitor. This might represent a previously unknown mechanism of action of graduated elastic compression stockings with interesting potential for deep vein thrombosis prophylaxis, particularly during surgical procedures that are performed with the patient in a reverse Trendelenburg position.

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The Lifeline Foundation of the Society for Vascular Surgery and the International Society for Cardiovascular Surgery, North American Chapter, invites grant applications for funding of meritorious research by young surgical investigators. The awards are intended for surgeons who have completed their formal surgical education in general surgery and who have completed or are in an advanced training program in vascular surgery.

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Grant awards are not intended to supplement salary, which will remain the responsibility of the institution in which the awardee holds an appointment. The awardee is expected to devote a significant amount of time to the funded project. A progress report must be presented to the Executive Committee of the Foundation by the following April 1, and, on completion of the project, a brief oral report is to be presented to the memberships of the two societies during a plenary session at the Joint Annual Meeting.

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