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Remodeling of the Rat Saphenous Vein Network in Response to Long-Term Gravitational Load

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Summary

Our main objective was to test whether chronic orthostatic body position induces network changes in the saphenous vein superficial tributary system of the rat. Fourteen male Sprague-Dawley rats were kept in tilted tube cages (45° head-up position) for two weeks to induce chronic gravitational load to their leg veins. Ten animals housed in normal cages and four animals kept in horizontally positioned tube cages served as controls. The whole superficial network of the left saphenous vein was microprepared surgically under anesthesia, superfused with saline and observed under a videomicroscope, while normal flow and pressure were maintained in the lumen. Branching angles, lengths of venous segments and their diameters were measured offline from digitized images using special image-analyzing software. Several branching angles at the popliteal confluence were significantly reduced by 12.5-15.8 %. The *in vivo* diameter of the main branch (936±34 *vs.* 805±44 μm) and of one of the popliteal tributaries (776±38 vs. 635±36 μm) increased ($p<0.05$), comparing vessels from tilted animals with those from normal controls. Maintaining the animals in horizontal tube cages did not induce the above alterations. The increased diameters and reduced branching angles of the saphenous vein network observed are adaptive responses of the venous network to a long-term gravitational load.

Key words
Mapping • Microcirculation • Remodeling • Veins • Gravitational load

Introduction

While the short-term effects of gravitational stress on the leg veins have been extensively studied (Terada and Takeuchi 1993, Puri and Segal 1994, Essfeld and Baum 1996, Vaitl *et al.* 1997), less is known about their chronic adaptation processes (Monos *et al.* 1989, 1991, 2001, Szentiványi *et al.* 1997). The failure of these processes or the pathologic sequels initiated by them can lead to the well-known varicose deformations. There is ample evidence that a chronic gravitational load forms the background of the human varicosity disease which is characterized by pathological alterations in the superficial tributary system of the saphenous vein (Zwillenberg *et al.* 1971, Alexander 1972, Mellmann *et al.* 1977, Leu *et al.* 1979, Thomas and Keeling 1986, Goren and Yellin 1990, Monos *et al.* 1995).

Most clinical investigations have focused on the extensive morphological, histological and biochemical changes found in the varicose deformations themselves. Human investigations analyzing the normal physiological adaptation processes of the saphenous vein tributary network to chronic gravitational load are difficult to devise for ethical and methodical reasons. A chronic tilt

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cage model was developed by us (Monos *et al.* 1989) to study experimentally changes induced by the chronic gravitational load in saphenous blood vessels of the rat. A morphological dilation of the vessel (Monos *et al.*1989), augmented myogenic response (Monos *et al.* 1991, Szentiványi *et al.* 1997), increased influence of sympathetic innervation on the smooth muscle membrane potential (Monos *et al.*1989), as well as increased density of adventitial nerve terminals and synaptic vesicles in the vessel wall (Monos *et al.* 2001) have been described.

The gravitational load and the consequently altered venous circulation may affect the whole tributary system of the saphenous vein. We have therefore investigated whether chronic gravitational load induces changes in the network characteristics of the superficial branching system of the rat saphenous vein, including branching angles, distances between confluences, vessel diameters, and disappearance or appearance of new branches.

Methods

Chronic tilt position of rats

The experiments were performed on male Sprague-Dawley rats. The investigation conforms with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). To induce chronic gravitational load on the leg veins, 14 animals were kept for two weeks 45° head-up position in tube-like cages. Earlier studies demonstrated that this body position doubles the pressure in the main trunk of the saphenous vein (Monos *et al.* 1989), and even higher pressure elevations can be expected in the more distal leg veins. The animals were able to move freely in the cage up and down on a built-in ladder, but they were not able to turn around, their head-up tilt position was continuously maintained. Rats had a free access to food and water at the upper end of the cage. Not to restrict their basic behavioral requirements, each day they were removed from the cage for an hour and allowed free movement and grooming in a normal rat cage. The room with the cages was temperature controlled. Weight of the animals, as well as food and water intake were daily measured. At termination of the two-week period the chronically tilted animals weighed 321±11 g, which was not significantly different from that of the control group (317±10 g). The control rats (n=10), which were kept similarly as the tilted ones in normal cages, were selected from their siblings. Keeping the animals in tilt cages resulted both in an increased gravitational load and movement restriction. In order to separate these two effects, four additional rats were kept in tube cages fixed in horizontal position (movement restriction without gravitational stress).

In vivo videomicroscopy of the superficial saphenous vein network

The functional morphology of the superficial tributary network of the saphenous vein was studied by *in vivo* videomicroscopy, using a specific preparation technique developed in our laboratory. The animals were anesthetized by intraperitoneal pentobarbital sodium (Nembutal, CEVA, 45 mg/kg) and taped onto an animal pad in a geometrically fixed position. Skin was opened and the whole superficial branching system of the left saphenous vein was exposed by careful micropreparation. Superficial veins down to the diameter of about 100 μm were prepared on the thigh, popliteal region and lower leg. Veins of the ankle and foot were not prepared. During preparation, fascia and connective tissue were removed from the vein wall at about 3/4 of the circumference, the connective tissue connection at the base was left intact. This kept the network in a fixed position. All exposed veins were superfused with warm perfusion solution, the composition of which was (in mmol/l): NaCl 119, KCl 4.7, NaH_2PO_4 0.89, $MgSO_4$ 1.17, $NaHCO_3$ 24.0, $CaCl_2$ 2.5, glucose 5.0, and EDTA 0.026. It was bubbled with 95 % O_2 and 5 % CO_2. The temperature was set at 37 ºC, the pH was 7.4.

The preparations were checked under preparation microscope. Only preparations devoid of strictures and thromboses were used. No signs of local venous flow failure were seen (edema, distension of veins). After one hour of incubation and careful superfusion with warm saline, the whole network was recorded with a videomicroscope using different magnifications (12.1-70.5x at the screen). A Wild M3Z stereomicroscope, equipped with a 2.0x standard objective, zoom levels set at 6.5-40x, a Philips videocamera and monitor, an Akai videorecorder, and a Volpi cold light fiberoptic illuminator comprised our videomicroscopic system.

Network analysis

The videotapes were replayed off-line, selected characteristic images of the network were frozen, digitized and stored. An image analyzing software developed by us was used to evaluate the digitized pictures. Distances and angles identified on the screen were marked with colored cursors that could be handled by the mouse and their values were displayed and stored. The spatial resolution was about 3 μm at high magnification. Accuracy was better than 1 % for all levels of optical zooming. Reproducibility of distance measurements, which included intraindividual variability of positioning the markers at the vein contours, was always better than 1.5 % (± standard deviation). The network values of tilted and untilted animals were compared using Student's unpaired t-test. In one case variations were compared with the F test. $P<0.05$ was considered as criteria for significant difference.

Table 1. Geometrical parameters of the superficial saphenous vein network of the left leg of rats kept for two weeks in normal cages (n=10) and in tube cages ensuring chronic 45° head-up tilt of the animals (n=14). The network was carefully prepared in anesthetized animals and videomicroscoped with normal pressure and flow in the lumen.

		Kept in control cages	**Kept in 45° head-up tilt tube cages**
	a	805 ± 44	936 ± 34*
	c	551 ± 34	597 ± 27
Diameter (μm)	f	635 ± 36	776 ± 38*
	……………………	……………………...	…………………...
	$a^3/(c^3+d^3+e^3+f^3)$	1.29 ± 0.09	1.25 ± 0.25†
Distances between origins of branchings (μm)	ch	2196 ± 464	1620 ± 378
	hg	4053 ± 644	3885 ± 635
	cf	69.4 ± 4.9	58.4 ± 2.3*
	de	47.1 ± 2.5	41.2 ± 2.1*
Angles between branches (degrees)	fj	108.7 ± 9.4	96.8 ± 4.4
	ah	58.8 ± 1.4	50.1 ± 3.8*
	ag	50.2 ± 2.6	44.6 ± 5
	ci	67.3 ± 5.1	68.5 ± 3.3

*For further explanation of the symbols marking morphologically stable branches see Fig. 3. Data are means ± S.E.M. * indicates significant differences between the two group of animals (p≤ 0.05 with the unpaired Student's t test), whereas † means significant difference between the scatter of the two groups (p≤ 0.05 with the F test).*

Results

The image of the popliteal saphenous vein confluence in a control rat taken with two different magnifications is shown in Figures 1 and 2. Tilting did not induce the appearance of new branches or disappearance of preexisting ones in the whole network studied. The sketch of the network with all morphologically stable side branches and the mean values of characteristic distances and angles are depicted in Figure 3 and Table 1. Corresponding values for the chronically tilted rats are also shown. The branching angles of the popliteal confluence altered significantly by 12.5-15.8 % as a result of chronic head-up tilting: the angle between the two outer and two inner branches were reduced. A similar change was found in the branching angle of the first medial side-branch ($p<0.05$). Two other branching angles in the popliteal neighborhood were also reduced, but this did not reach the level of statistical significance. The diameter of the main branch and of the lateral side branch of the popliteal confluence was increased in tilted rats compared with the controls by 16.2 and 22.2 %, respectively ($p<0.05$). Alterations in other measured distances and diameters did not reach the level of statistical significance. For the popliteal confluence (four branches connecting the main branch) a constant shear ratio was computed. This parameter, the cube of the main branch divided by the sums of cubes of all side branches, should have the value of one, if the shear induced by blood flow at the walls of all affected vessels was constant (Rodbard 1975, Rossitti and Lofgren 1993). Its value was found somewhat above one and was similar in both groups despite the alterations of the corresponding branches (Table 1). However, the variability of this parameter was found to be significantly higher in case of chronically tilted rats.

A similar difference between branching angles was found when head-up tilted animals were compared with those kept in horizontal tube cages.

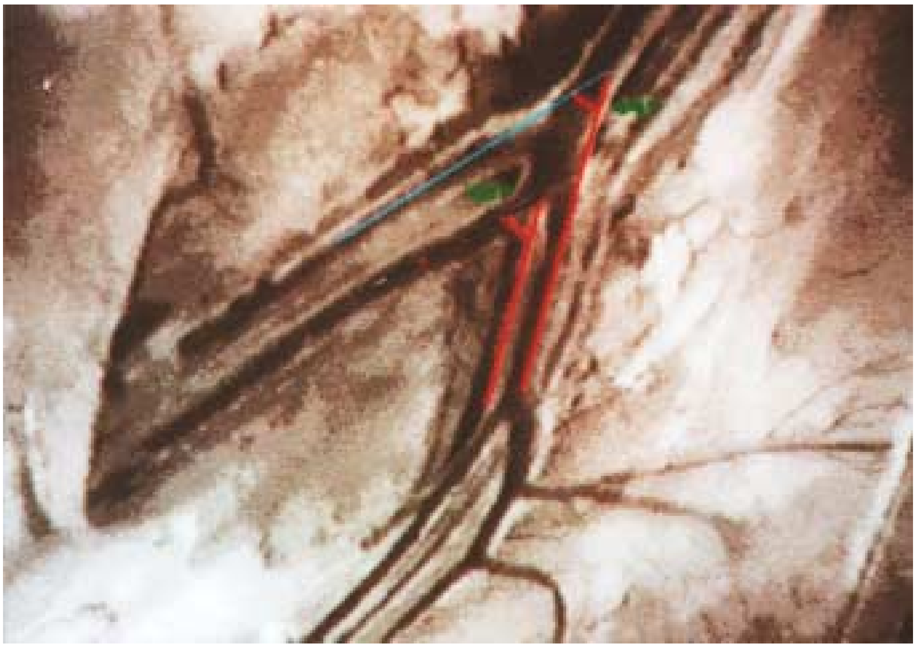

Fig. 1. *The popliteal confluence system of the rat saphenous vein (10x magnification). In vivo videomicroscopic picture of the in situ microprepared network. Normal pressure and flow in the lumen are maintained. Angle measurements at the outer and inner branches of the confluence system are shown.*

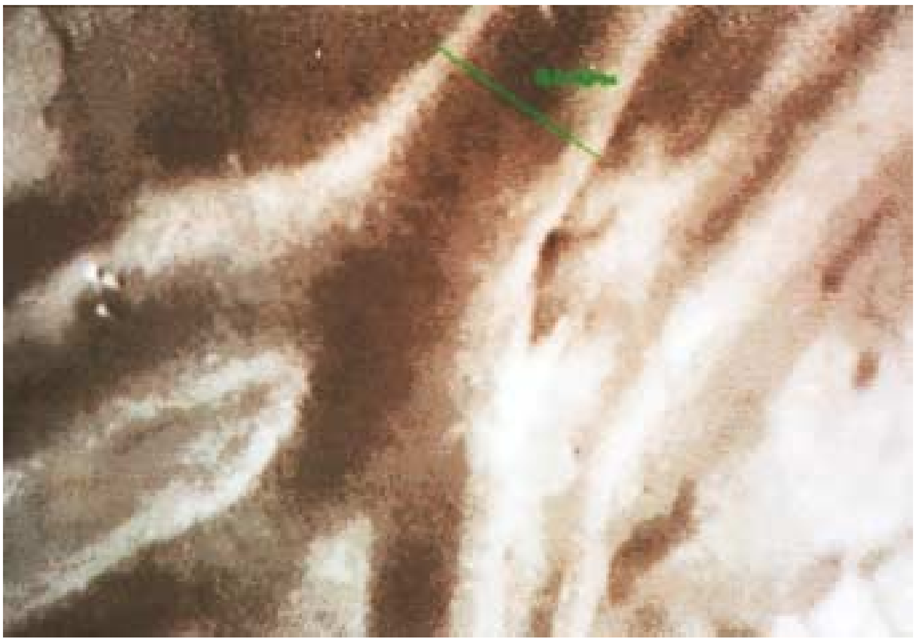

Fig. 2. *The same popliteal confluence system as in Fig. 1. with higher magnification (40x). Diameter measurement at the parent vessel is shown (814 µm in this case).*

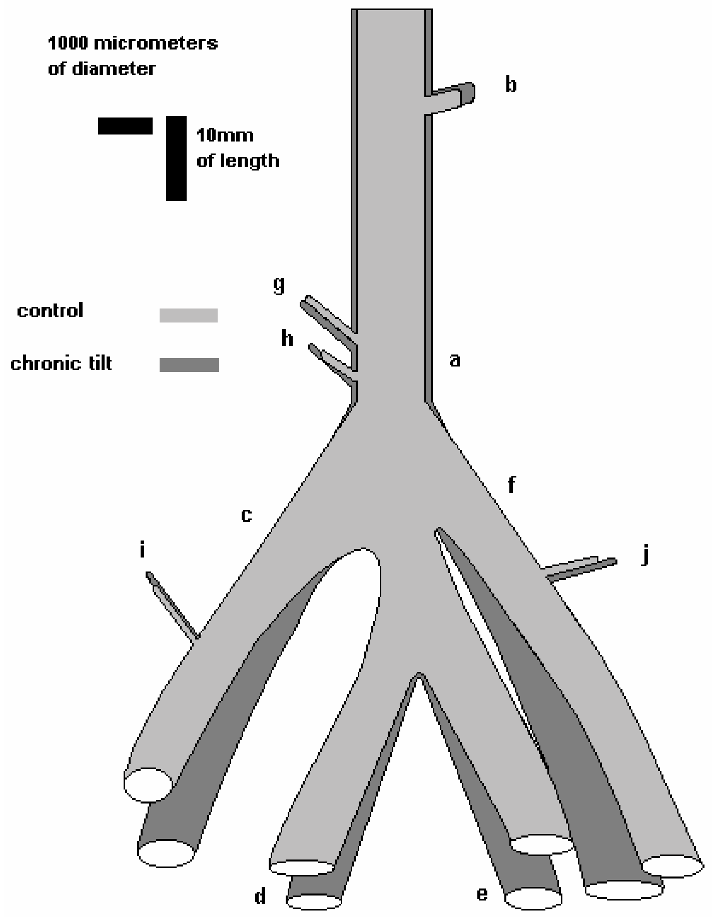

Fig. 3. *Alterations in the superficial saphenous vein network of the left leg of the rat following two weeks of orthostatic load. The drawing shows the morphologically stable branches (marked with letters a through j), their typical position in the network and typical alterations induced by keeping the animals in the chronically tilted position. Note that scales for lengths and diameters are not identical.*

Discussion

Two types of significant network alterations were observed in this study, as a result of head-up tilt body position for two weeks. One is the dilation of the *in vivo* diameter, affecting the main branch and one tributary of the popliteal confluence. The other concerns the reduction of branching angles, which affected all components of the popliteal confluence and a branch nearby. This chronic gravitational loading did not induce alteration of the basic pattern of the superficial saphenous vein network of the rat lower limb, because no appearance or disappearance of whole branches were observed among vessels over the 100 μm diameter range.

The *applied in vivo videomicroscopic technique* provides a possibility of studying the outer diameters of all components of the rat saphenous vein tributary system *in vivo*. Fixation of the leg in a geometrically reproducible position makes the measured angles comparable for all rats. There was evidently an undisturbed blood flow in all our preparations. However, some deviations from the normal *in vivo* situation can be expected as a result of removal of surrounding connective tissue from the veins. Support from surrounding tissue is an important component of venous mechanics at lower pressures. Another factor is that perivascular sympathetic fibers were injured during preparation. Anesthesia and fixing the leg in a semi-rigid position reduces the effect of the muscle pump. Washing the surface with oxygenized saline eliminates the effects of vasoconstrictor and vasodilator factors released from neighboring tissues, and even blood-borne vasoactive factors are washed out at a higher rate from the venous wall. Despite these limitations, this technique allows to study the effects of network morphogenesis in living preparations.

A morphological *dilation of the saphenous vein* main branch after two weeks of head-up tilting has also been found in an earlier *in vitro* study (Monos *et al.* 1989). It has now been confirmed under *in vivo* conditions, and has also been shown that other branches of the saphenous vein superficial tributary system are affected as well. The mechanism of this morphological venodilation is not clear. It is also possible that a passive distension induced by chronically elevated venous pressure which is stabilized morphologically, as it has been supposed for the development of the human varicosity disease (Zwillenberg *et al.* 1971, Alexander 1972, Leu *et al.* 1979, Goldman and Fronek 1989, Clarke *et al.* 1992, Monos *et al.* 1995). Another factor involved is that the leg blood flow to hypertrophized muscles is elevated (Hudlicka *et al.* 1995) and the consequently enhanced endothelial shear dilates the veins. From this point of view it is noteworthy that the equal shear ratio was close to the unit and did not change despite significant elevation of the diameter of affected veins. This suggests that endothelial control of the morphological lumen may have been involved both in tilted and untilted animals. The significantly higher scatter of the shear ratio in tilted animals marks some disturbance in flow control of vessel diameter in these animals, which might have been induced by augmented venomotion described earlier (Puri and Segal 1994).

The other characteristic change induced by chronic tilting was *a reduction of branching angles*, which affected branching angles in the neighborhood of the popliteal confluence. These data prove that even a two week-long tilt period was sufficient for a partial remodeling of the whole network. As to the function of this remodeling we do not have sufficient objective data available at present. Energetic optimization seems to be in the background of the formation of branching angles of cerebral arteries (Rossitti and Lofgren 1993). The affected venous segments could have moved in a lateral direction in-between the layers of their connective tissue sheaths. Another possibility is that they have followed passively the rearrangement of the fascia in which they were ensheathed. The displacement of the site of mounding of the side branches along the axis of the parent vessel could be excluded, at least in the case of the two medial side branches of the main trunk above the popliteal region.

In conclusions, significant geometrical alterations occur in the network properties of the superficial leg veins of the rat when subjected to two weeks of experimental orthostatic load. Branching angles forming the popliteal confluence and some in its neighborhood were altered. The observed network alterations seem to be components of adaptive processes to chronic gravitational load. The described gravitational venodilation and the lateral displacement of venous segments resemble some of the pathological alterations observed in human varicosity disease.

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