

**EFFECTS OF POSTURE AND VENOUS INSUFFICIENCY ON  
ENDOTHELIAL-DEPENDENT AND -INDEPENDENT CUTANEOUS  
VASODILATION IN THE PERIMALLEOLAR REGION**

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Short Title: Venous insufficiency and vasodilator function

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## **Abstract**

**Objectives:** To assess the effects of posture, endothelial function and venous insufficiency on cutaneous microvascular vasodilator function in the gaiter area, in particular defining factors which may affect microangiopathy and ulcer formation.

**Methods:** Endothelial-dependent and –independent vasodilator responses to incremental-doses of acetylcholine (Ach) and sodium nitroprusside (SNP) were evaluated in the perimalleolar region in the supine and standing positions in middle-aged patients with isolated superficial venous insufficiency (ISVI) (n=25) and health controls (n=28) using laser Doppler fluximetry (LDF) and iontophoresis of vasodilators.

**Results:** The venoarteriolar reflex (vasoconstriction on standing) was equally present in both groups, and reduced the vasodilator responses to SNP in the upright position (e.g for patients with ISVI, peak SNP response was  $82 \pm 11$  PU [standing] vs  $123 \pm 15$  PU [supine]). The presence of ISVI had no effect on endothelial vasodilator function in the supine position, but on standing cutaneous reactivity to Ach was significantly reduced (e.g peak Ach response  $69 \pm 8$  PU [ISVI] vs  $109 \pm 11$  PU [controls],  $p < 0.003$ ).

**Conclusions:** Upright posture impairs cutaneous endothelial-dependent vasodilation in the gaiter area of patients with ISVI. This may be of clinical and prognostic utility in identifying which patients with uncomplicated ISVI are at highest risk of tissue breakdown and ulcer formation in the gaiter area.

**Key Words:** Endothelial function; microvascular; venous insufficiency; ulceration;

## **Introduction**

Gravitational ulceration around the gaiter area of the lower limb (above the medial or lateral malleolus) due to primary venous insufficiency is a common disabling condition that is difficult to treat once established and prone to recurrence [1]. The underlying mechanisms of tissue breakdown and ulcer formation are complex and not clearly understood, but microangiopathy involving structural and functional abnormalities is an established feature of venous hypertension [2] and a possible target for therapeutic intervention [3].

Tissue viability and susceptibility to ulceration depend upon many factors, but cutaneous microvascular perfusion and in particular the integrity of compensatory vasodilator mechanisms to maintain blood flow, tissue oxygenation and nutrient delivery despite changes in systemic and local haemodynamics may be especially important [4]. For example, it has been shown that reduced vasodilator capacity in response to warming correlates with low transcutaneous oxygen tension in the perimalleolar tissues [5], but very little is known about how microvascular vasorelaxation is dependent upon endothelial function, posture (i.e neural reflex pathways) and/or defects in the underlying vascular smooth muscle.

Although laser techniques have been available for many years, recent technological improvements in laser Doppler fluximetry (LDF) and the associated analytical software offer more reliable methods and applications for studying microvascular vasodilator pathways in clinical studies, e.g by combining LDF with iontophoretic administration of vasoactive drugs [6]. Thus, the purpose of the present study was to compare cutaneous microvascular perfusion around the gaiter area in the supine and

upright positions in patients with isolated superficial venous insufficiency (ISVI) and normal healthy subjects, and to assess the effects of ISVI and posture on both endothelial-dependent and endothelial-independent vasodilator mechanisms.

## **Methods**

Patients with uncomplicated ISVI, i.e long or short saphenous vein reflux confirmed by Duplex scanning, were recruited from referrals to the Vascular Laboratory at Derbyshire Royal Infirmary. Patients with present or past venous ulceration, arterial disease, or major skin changes in the gaiter area were excluded. Healthy control subjects were recruited from the research database in the Division of Vascular Medicine, and the absence of ISVI among control subjects was confirmed by Duplex scanning. All volunteers provided written informed consent, and a detailed clinical protocol was approved by the Southern Derbyshire Research Ethics committee. Following a baseline screening visit, which included measurements of height, weight and BP, each subject attended the Clinical Research Unit for a single study morning undertaken in a temperature-controlled room according to a standard protocol.

### *Study periods*

Patients acclimatised to the room during a 30min period of supine rest with the leg to be studied supported at a 30° angle. The gaiter area was cleaned with alcohol and dried thoroughly before applying two perspex iontophoresis chambers (Moor Instruments Ltd, Axminster, UK) to the surface of the leg 4-8 cm proximal to the medial malleolus. The chambers were positioned over healthy looking skin, approx. 2-5 cm apart. 0.25ml of 1% acetylcholine (Ach) (Sigma Chemicals, UK) and 1% sodium nitroprusside (SNP) (Nipride, Roche Pharmaceuticals Ltd) diluted in

deionised HPLC-grade water was then injected into the anodal and cathodal iontophoresis chambers, respectively, and a laser Doppler probe positioned through the centre of each chamber. The chambers were no more than 4cm apart and positioned over healthy skin (avoiding any area of lipodermatosclerosis or superficial veins). After achieving a stable recording of baseline flux, LDF responses to transcutaneous administration of the endothelial-dependent vasodilator Ach and the endothelial-independent vasodilator SNP were measured using an incremental-dose iontophoresis protocol [7]. Thereafter, following 10 mins recovery and stabilisation of baseline flux recordings in the standing position, repeat measurements of vasodilator responses were performed with the subject upright.

The DRT4 LDF system was utilised for all studies (Moor Instruments, Axminster, UK), together with the Moor Instruments iontophoresis controller and the DRT4 software for automated data analysis of skin temperature, flux and microvascular dose-response curves for each iontophoretic challenge.

#### *Incremental microvascular dose-response analysis*

The principle of drug iontophoresis is that an electrical potential difference will actively cause ions in solution to migrate according to their electrical charge. The magnitude of the electrical charge ( $Q$ ) is therefore dependent upon length of time ( $t$ ) a current ( $I$ ) is passed (i.e  $Q = It$ , where charge is quantified in coulombs, current in amps and time in seconds). Thus, duration and current influence the dose of agonist that is delivered.

In this study, the iontophoresis controller (Moor Instruments) delivered low electrical currents of preset intensity for a specified duration. Each current stimulus was followed by a period of laser Doppler recording to monitor the time-dependent microvascular flow response. Following a stable baseline recording, dose-response curves for Ach and SNP induced vasodilation were characterised using incremental charges according to a standard protocol, as described previously [7]: 25 $\mu$ A for 10s (ie 250 $\mu$ C), 50 $\mu$ A for 10s (500 $\mu$ C), 100 $\mu$ A for 10s (1000 $\mu$ C), and 100 $\mu$ A for 20s (2000 $\mu$ C), with a 4-min recording period between each electrical charge.

#### *Statistical analysis*

A LDF commercial software system (Moorsoft V1, Moor Instruments, UK) was used to provide automated analysis of the microvascular Doppler flow responses to incremental doses of each agonist, and the data (maximum perfusion measured in Perfusion Units [PU]) for individual subjects was then downloaded to a PC for comparison between groups using the non-parametric Mann-Whitney U-test and within groups using ANOVA for repeated measures. Both Clinical data and the vasodilator responses for each incremental charge are presented as mean  $\pm$  SEM.

## **Results**

Twenty-five patients with ISVI and 28 control subjects without ISVI participated in the study. The clinical and demographic details are presented in Table 1.

#### *Effects of posture on endothelial-dependent & -independent vasodilator responses*

In control subjects, rising from the supine to the standing position was associated with a significant reduction in baseline cutaneous flux at both perimalleolar probes (Figure

1), typical of the normal venoarteriolar response to upright posture [8]. However, this reduction in cutaneous perfusion appeared to be fully overcome by the endothelial-dependent vasodilator Ach: peak microvascular responses to Ach were similar in the standing compared with the supine position (Figure 1). In contrast, perfusion responses to each dose of SNP were attenuated in the standing position (Figure 1).

A similar venoarteriolar reflex was also demonstrated in patients with ISVI: baseline cutaneous perfusion was significantly reduced when patients moved from the supine to the standing position, and the upright response to SNP was blunted (Figure 2). However, in contrast to the healthy controls, stimulation with Ach did not overcome the effect of posture on cutaneous perfusion: Ach-induced vasodilation was significantly reduced in the standing position (Figure 2).

#### *Effects of ISVI on endothelial-dependent and –independent vasodilator responses*

Baseline (resting) cutaneous perfusion in the gaiter area, both in the supine and standing positions, was no different in patients with ISVI compared with controls. With subjects supine (i.e without the venoarteriolar reflex active) endothelial-dependent vasodilation to incremental doses of Ach was similar in patients vs controls (e.g peak Ach response was  $105 \pm 9$  PU [ISVI] vs  $121 \pm 11$  PU,  $p=0.27$ ) (Figure 3), whereas the peak vasodilator response to SNP was significantly lower in the presence of venous disease ( $123 \pm 15$  PU vs  $152 \pm 12$  PU,  $p<0.05$ ) (Figure 3).

When subjects moved to the standing position and the venoarteriolar (vasoconstrictor) reflex was activated, there was no difference in vasodilator responses to SNP between the two groups, i.e endothelial-independent vasodilation was equally blunted in

patients and controls. However, the endothelial-dependent vasodilator response to Ach in the upright position was significantly lower in patients with ISVI ( $69 \pm 8$  PU vs  $109 \pm 11$  PU,  $p < 0.05$ ) (Figure 3).

## **Discussion**

This study provides several important observations about the inter-play of endothelial function, posture and vascular smooth muscle tone in the regulation of microvascular function in control subjects and in patients at risk of perimalleolar ulceration due to lower limb venous insufficiency. Firstly, in the supine position resting cutaneous perfusion in the gaiter area was similar in patients with ISVI compared with controls, and the two groups showed similar incremental responses to the endothelial-dependent vasodilator Ach. Absolute skin flux, even in healthy individuals, is lower in the gaiter area compared with the shin or the dorsum of the foot [9], which no doubt explains in part why this region is so vulnerable to tissue breakdown. Although resting perfusion is often increased over areas affected by lipodermatosclerosis or ulceration [10], the result of this study contrasts with earlier suggestions that even mild venous insufficiency is associated with cutaneous hyperaemia [11].

Whereas Ach-induced vasodilation in larger conduit vessels is mediated almost entirely by endothelial release of nitric oxide (NO), the mechanisms involved in cutaneous microvessels are less clear. It seems likely, however, that Ach-induced release of vasodilator prostanoids and/or endothelium-derived hyperpolarising factor (EDHF) is more important than NO [12]. Thus, under conditions of supine rest and standardised temperature-control there was no evidence of impaired endothelial-

dependent vasodilation in patients with ISVI. A similar result was reported after iontophoretic administration of a single-dose of pilocarpine [13]. These observations are especially relevant to the on-going debate about what causes ISVI, in particular suggestions that endothelial dysfunction and venous dilatation (rather than valve failure) might be primary mechanisms in the pathogenesis of varicose veins [14].

Cutaneous vasoconstriction in response to standing is often referred to as the venoarteriolar reflex (or 'oedema protection reflex') [8]. Local neuro-vascular mechanisms, including the sympathetic nervous system, cause vasoconstriction in response to venous hypertension and thus buffer the rise in capillary pressure and transudation of fluid into the surrounding tissues. There have been various reports that the venoarteriolar reflex is either intact [15] or impaired [16] in patients with ISVI, the latter possibly due in part to a form of neuropathy affecting small unmyelinated C-fibres in patients with venous disease [17]. In the present study, however, ISVI had no effect on the normal vasoconstrictor response to standing.

In control subjects and in those with venous insufficiency, the venoarteriolar reflex effectively blunted the vasodilator response at all doses of SNP, presumably by increasing background sympathetic vasoconstrictor tone on vascular smooth muscle. This would be consistent with noradrenergic mechanisms, activated by standing, opposing the direct vasorelaxant effect of SNP on vascular smooth muscle. However, it notable that upright responses to SNP were equally blunted, relative to supine responses, in both patients with ISVI and controls, i.e this effect of posture was not augmented or diminished by coexistent venous disease.

There were interesting differences between the groups with respect to Ach-induced vasodilation in the upright position. Firstly, in healthy subjects, Ach stimulation completely reversed the effect of the venoarteriolar reflex: peak vasodilator responses to Ach in the standing position (with the venoarteriolar reflex activated) were almost identical to those in the supine position (when the reflex was not active). This was not the case in patients with ISVI, where maximal Ach-induced vasodilation was significantly lower in the upright compared with the supine position. Several observations can be made about this result. Firstly, from the baseline flux measurements and the SNP data it seems unlikely that the venoarteriolar reflex was increased in patients with venous insufficiency. Instead, Ach-induced release of EDHF from the microvascular endothelium, and/or EDHF responsiveness of the vessel wall, may be reduced under conditions of raised venous pressure.

Thus, the present study has shown that there is impaired endothelium-dependent vasodilation around the gaiter area in patients with ISVI when venous pressure is high, e.g in the upright position. This suggests that standing is associated with impairment of a compensatory mechanism which is probably important for maintaining tissue perfusion and viability in venous insufficiency. Further studies should address whether impaired Ach-induced vasodilation in the upright position is of prognostic utility, perhaps providing a non-invasive measure of high ulcer risk among patients with uncomplicated ISVI.

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## Figures

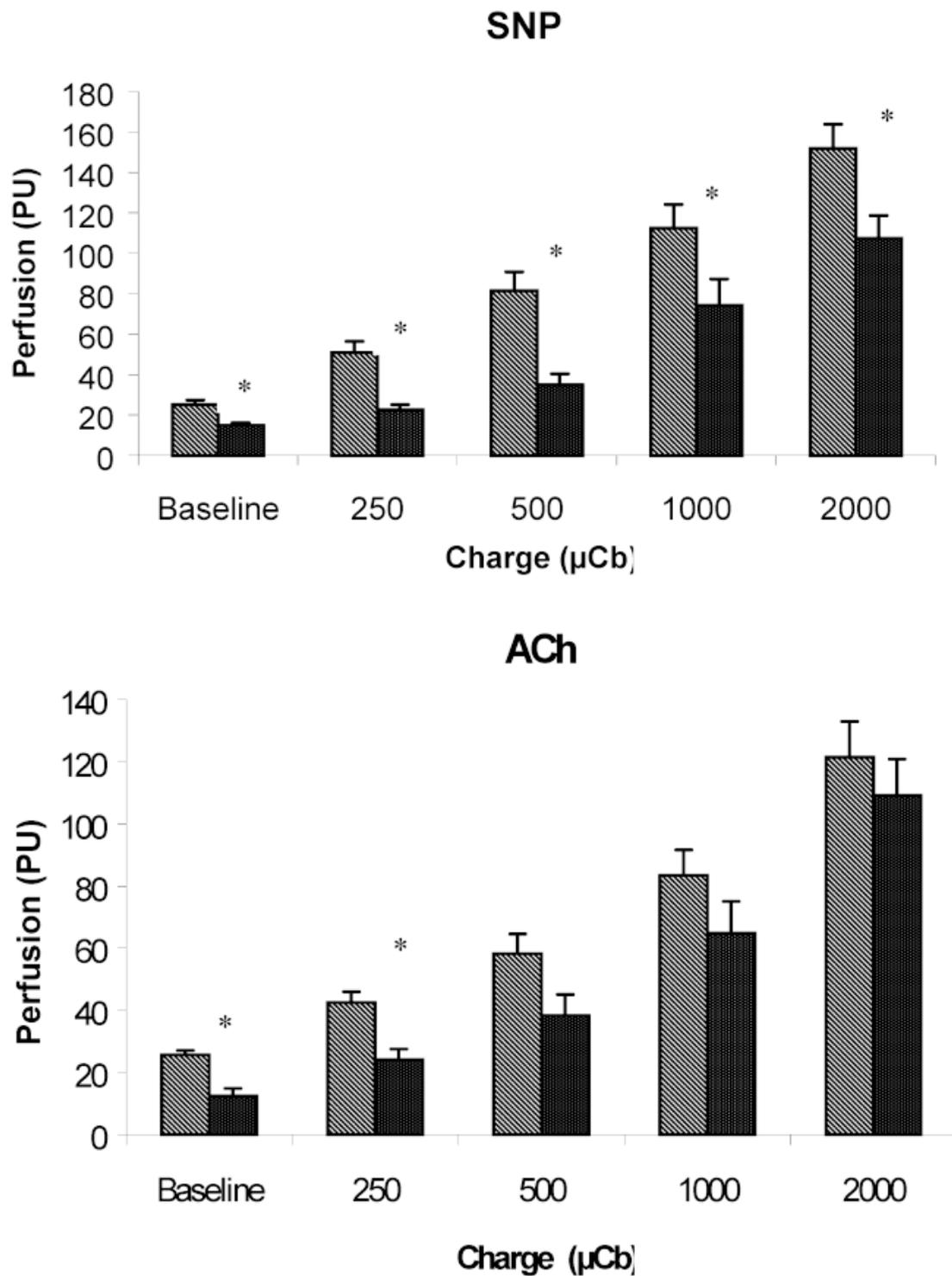


Figure 1: Cutaneous microvascular vasodilator responses to incremental doses of SNP (upper panel) and ACh (lower panel) in healthy control subjects (n=28) in the supine [ ▨ ] and standing [ ■ ] positions; \* p<0.005.

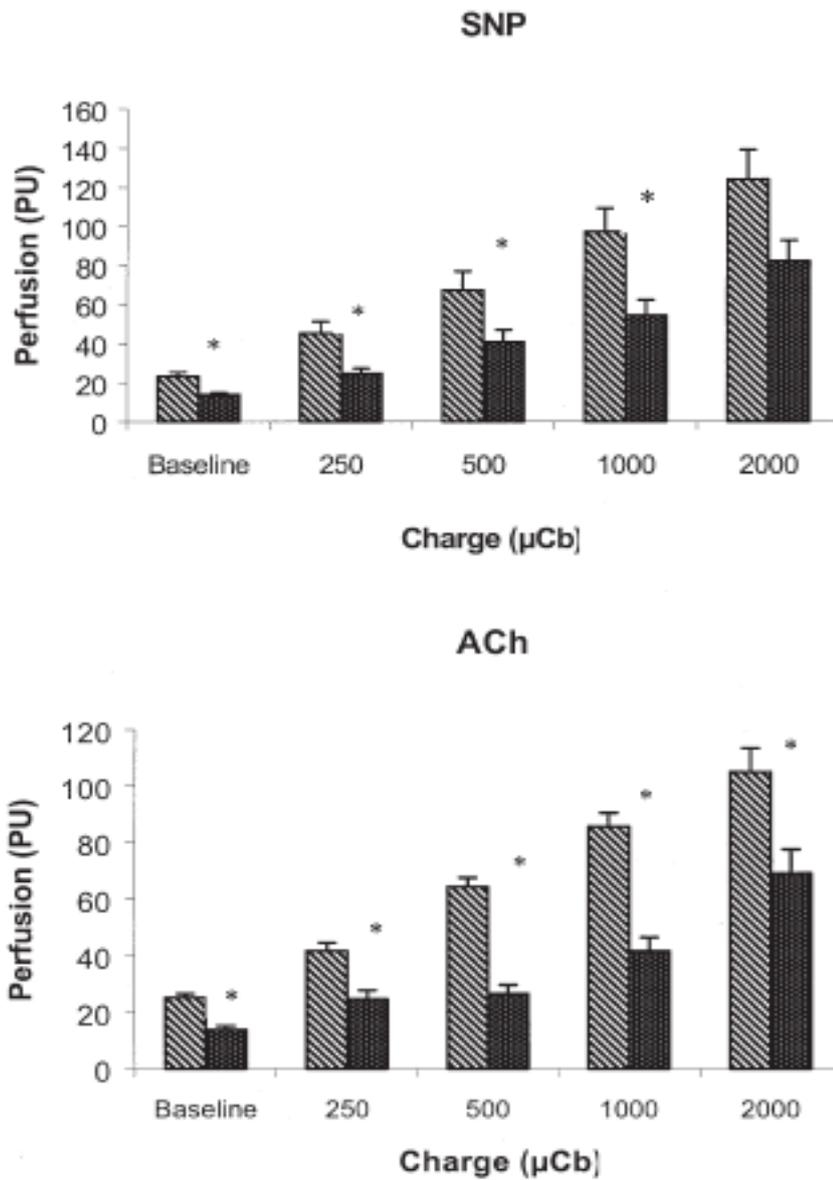


Figure 2: Cutaneous microvascular vasodilator responses to incremental doses of SNP (upper panel) and Ach (lower panel) in patients with ISVI (n=25) in the supine [ ▨ ] and standing [ ■ ] positions; \* p<0.005.



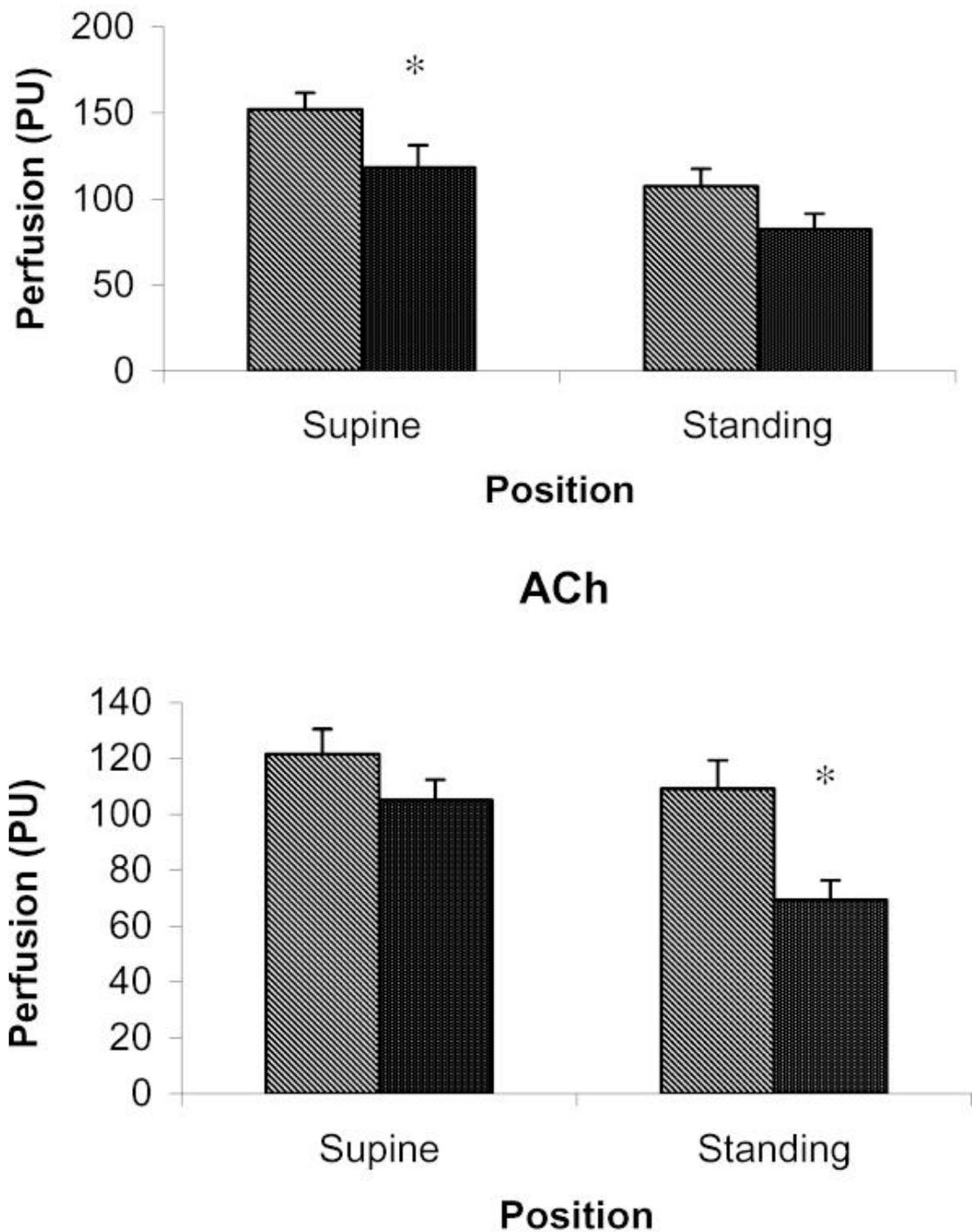


Figure 3: Comparison of peak vasodilator responses to SNP (upper panel) and ACh (lower panel) in patients with ISVI [ ■ ] compared with controls [ ▣ ] in the supine and standing positions. \*  $p < 0.05$  between groups.



**Table 1****Clinical & demographic features of study groups. Mean & (SEM)**

	<b>Healthy Controls (n=28)</b>		<b>Isolated Superficial Venous Insufficiency (n=25)</b>	
	<b>Mean</b>	<b>Range</b>	<b>Mean</b>	<b>Range</b>
<b>% Female</b>	60%		52%	
<b>Age (Years)</b>	48(11)	31 - 72	57(11)	31 – 80
<b>Weight (kg)</b>	75(13)	54 - 108	79(14)	49-100
<b>Height (cm)</b>	168(0.08)	157 – 188	170(0.08)	160-183
<b>Systolic Blood Pressure (mmHg)</b>	125(13)	104 – 153	140(25)	103-180
<b>Diastolic Blood Pressure (mmHg)</b>	80(10)	62 – 97	82(10)	67-97

