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The direct effect of leg position on calf blood flow measured by venous occlusion plethysmography[☆]

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Summary *Background:* Venous occlusion plethysmography is commonly used to assess changes in calf blood flow (CBF). Although the leg is often positioned above the level of the heart to aid venous emptying during periods of cuff deflation, its direct effect on measured CBF is not known. We therefore planned to determine if CBF is affected by raising the calf region at the same body position during constant vasoconstrictor sympathetic nerve drive and haemodynamic variables.

Methods: We measured concomitant heart rate, arterial pressure, muscle sympathetic nerve activity (MSNA), calf blood flow (CBF) and calf vascular resistance (CVR) in the semi-supine position with the leg supported by the heel at various elevations above the horizontal level.

Results: In 26 subjects we found that raising the leg to $40 \pm 0.65^\circ$ significantly increased CBF by about $23 \pm 4.7\%$ at constant haemodynamic variables and MSNA levels. Furthermore, in 10 of the 26 subjects this effect was graded within the same constant conditions. When the calf region was elevated to two positions at $22 \pm 3.5^\circ$ and $40 \pm 1.5^\circ$ from the horizontal level the increase in CBF, respectively, amounted to $13 \pm 5.9\%$ and $37 \pm 5.9\%$.

Conclusions: It was shown that measurement of CBF by strain gauge venous occlusion plethysmography is directly affected by the position of the calf region above the horizontal level. It is suggested that this could confound measurement of calf blood flow in longitudinal and interventional studies.

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Introduction

Venous occlusion plethysmography is an undemanding and non-invasive method that is readily available for the measurement of limb blood flow.^{1–5} The technique has been based on the measurement of calf volume changes

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in response to venous occlusion by a cuff with the volume changes in a limb reflecting the rates of arterial inflow. In order to facilitate adequate venous emptying during periods of cuff deflation, the limb is usually positioned above the level of the heart by resting it at the heel on a foam support. In addition, in order to enable assessment of blood flow supplying mainly the calf muscle region (CBF), a mercury sphygmomanometer cuff is usually applied to the ankle and inflated to levels above systolic arterial pressure to prevent arterial blood flow distally into the foot.

This method has been widely used to examine biological changes in calf blood flow (CBF), making it important to determine beforehand the errors attributed to venous occlusion plethysmography itself.^{1–3,5–8} The latter has therefore been assessed using within-subject serial measurements of limb flow under standardized conditions to exclude biological interference. These conditions have included maintenance of the same body position, avoiding artifacts during acquisition of recordings, and similar placement of cuff and strain gauge positioning.^{3–5,9,10} However, there has been no information available on the influence of leg elevation above the horizontal level on measured CBF at constant haemodynamic variables and vasoconstrictor sympathetic neural drive that can affect the vessels of the calf region.

This study was therefore undertaken to quantify in individual subjects the effect of leg position on the measurement of CBF at the same semi-supine body position, muscle sympathetic nerve activity (MSNA) supplying calf blood vessels and haemodynamic variables.

Methods

Subjects

A group of 26 subjects with a wide range of age, body mass index and haemodynamic variables was examined. They were recruited for a study investigating the relationship between changes of the peripheral sympathetic drive and their effect on hypertension. Of these 26 subjects, hypertension was diagnosed in 22 and was excluded in the remaining four subjects. The latter volunteered for the study, and were included within the overall within-subject analysis because they exhibited similar results to those in the former group. All subjects underwent a screening medical history and examination before the study, and none was on medication for medical conditions. They were excluded if there was evidence of varicose veins, edema, peripheral vascular disease, autonomic or peripheral somatic neuropathy and other chronic disease that may influence peripheral sympathetic nerve activity and its vascular effect. The investigation was conducted according to the principles of the Declaration of Helsinki (2000) of the World Medical Association and carried out with the approval of St. James's University Hospital Ethics Committee, with all subjects providing informed written consent.

General protocol

Calf blood flow (CBF), haemodynamic variables and muscle sympathetic nerve activity (MSNA) were simultaneously

measured over a 2 min period during the steady state. The details of the protocol of each session and data analysis have been published previously.^{11–13} Briefly, all investigations were performed under similar conditions between 09.00 and 12.00 hours. Participants were asked to have a light breakfast and to empty their bladder before commencing the study. They were requested to avoid nicotine, caffeine, alcohol and strenuous exercise for 24 hours prior to investigation.

During each session, the subjects were studied in the same semi-supine position, which was not changed during leg raising to avoid head-up or head-down tilt-induced changes in haemodynamic variables and their reflex effects. Measurements were made in a darkened laboratory in which the temperature was constant at 22–24 °C. Resting arterial pressure was measured from the upper arm using a standard mercury sphygmomanometer as this was similar to that obtained from the thigh. Heart rate and arterial pressure were monitored and recorded using an electrocardiogram and a Finometer device (FMS, Arnhem, The Netherlands), respectively. MSNA was measured by microneurography and blood flow to the muscle of the left calf was obtained using standard venous occlusion strain gauge plethysmography.

Measurements were taken in a random order with the calf at the lowest or highest position whereby the middle of the calf at the strain gauge placement, respectively, was about 7 cm or 21 cm above horizontal level. These two positions, respectively, corresponded to 5° and 40° from the horizontal level relative to hip position as measured by a large international standard Goniometer. In some of the subjects, the effect was graded by using three randomly used leg positions with an intermediate height of about 13 cm (at an angle of about 20°), between the two aforementioned positions. Measurements at all positions were carried out only when the data had returned to steady state baseline values.

Microneurography

Post-ganglionic muscle sympathetic nerve activity (MSNA) was recorded from the right peroneal nerve for at least 2 min along with the other data, as previously described.^{11–13} All subjects attained steady state in respect of all measured variables for at least 30 min before recordings were made. Briefly, the neural signal was amplified ($\times 50,000$), and for the purpose of generating bursts representing multiunit discharge, the signal was filtered (bandwidth of 700–2000 Hz), and integrated (time constant 0.1 s). The output of action potentials and bursts from this assembly was passed to a PC-based data-acquisition system (LabVIEW, National Instruments Corp., Austin, TX, USA), which digitised the acquired data at 12,000 samples/second (16 bits).

MSNA was differentiated from skin sympathetic nerve activity and afferent traffic by previously accepted criteria.^{14,15} Only vasoconstrictor activity was accepted and examined. This was determined by observing appropriate responses to spontaneous changes in arterial pressure, the Valsalva maneuver and by a screening isometric handgrip exercise tests. During the Valsalva maneuver, sympathetic activity increased during the latter part of phase-II and/or phase-III and decreased during phase-IV

(corresponding to the decrease and increase of arterial pressure). The Valsalva maneuver was performed by exhaling into a standard mercury manometer, at a constant pressure of about 45 mmHg for approximately 18 s, and was monitored by a pneumograph. Isometric handgrip exercise was performed using a dynamometer, by employing 30–40% of a pre-determined maximal voluntary contraction, to confirm a late increase in MSNA.¹⁶

Analysis of nerve activity was performed off-line independently from other measured variables, using dedicated software based on the LabVIEW system (National Instruments Corp., Austin, TX, USA). An electronic discriminator window was used objectively to count the waves of MSNA bursts when the signal-to-noise ratio was >3 , and this was quantified as the mean frequency of bursts/100 cardiac beats to avoid any influence by the length of the cardiac cycle.¹⁷ The variability of measuring MSNA in this laboratory did not exceed 10%,¹¹ and the frequency of MSNA in the right peroneal nerve was similar to that in the left peroneal nerve as previously reported.^{17,18}

Plethysmography

CBF was obtained using mercury-in-silastic (Whitney) strain gauge venous occlusion plethysmography (D.E. Hokanson Inc., Bellevue, WA, USA). The strain gauge was placed around the widest circumference of the left calf region, and chosen to be 2–3 cm smaller than the calf circumference such that it was applied under slight tension to the calf. With participants in the semi-supine position, the leg was always supported by the heel on a foam support in order to avoid the strain gauge coming into contact with the bed and to aid venous return.

Venous occlusion was effected by inflating a contoured thigh cuff (Model CC-22, D.E. Hokanson Inc., Bellevue, WA, USA) placed around the left thigh to about 60 mmHg or 20 mmHg below a pre-determined diastolic arterial pressure, whichever was the lesser. This cuff had two wide bore ($\frac{1}{2}$ " diameter) inflation tubes, so that the cuff could be rapidly inflated to a pre-determined pressure within 0.5 s by using a rapid cuff inflation device (Model EC20, D.E. Hokanson Inc., Bellevue, WA, USA) and a compressed, dry air source (Linde Gas UK Ltd., West Bromwich, UK). The DC output from the plethysmograph was passed to a chart recorder (APC Medical Ltd., Welwyn Garden City, Herts., UK) utilising heat sensitive paper, so that a graphic record of change in limb volume could be produced. During measurement of CBF, the left foot region was excluded by inflating a pediatric cuff placed round the ankle to levels greater than a pre-determined systolic arterial blood pressure. With the strain gauge in situ, pre-measurement trials of venous occlusion were made to establish satisfactory and comfortable positioning of the cuffs and baseline recording positions. With the calf region occluded, venous occlusion was employed for approximately 5–10 s followed by rapid deflation. A period of time was allowed between venous occlusion to ensure that the limb volume changes returned to baseline levels.

During each CBF measurement, at least six recordings were made over the period of 2 min, and the value of CBF represented the average of all these measurements. Each CBF was obtained from the graphical plethysmographic

recordings. The initial slope of the volume curve was measured after ignoring any inflation artifact, and was then used to derive the rate of volume increase; CBF was expressed in units of ml of flow per 100 ml tissue per min ($\text{ml } 100 \text{ ml}^{-1} \text{ min}^{-1}$). The intra-observer reproducibility of CBF measurement in this laboratory, obtained as twice 95% confidence interval of differences between repeated within-session plethysmography, amounted to 2.4% of the value of the measurement. Arterial pressure was simultaneously and continuously measured during the 2 min and its average value was divided by the average CBF to obtain calf vascular resistance (CVR), which was expressed in arbitrary units. The variability of repeatedly measuring CBF within one leg or between the two legs in this laboratory did not exceed 15%.^{6–8}

Statistics

Within-subject changes of measured variables were assessed using Student's *t* tests for paired variables. Within-subject changes spanning more than two measurements were examined using repeated measures analysis of variance (ANOVA) with Newman–Keuls post-tests. The least square technique was used for assessing the linear relationship between variables. Values of $P < 0.05$ were considered statistically significant. Data are presented as mean \pm SEM.

Results

Of the 26 subjects there were 11 women and 15 men, and their ages were 48 ± 2.6 years (range 24–71). Their body weight and body mass index, respectively, were 83 ± 2.4 kg (range 59–105) and $29 \pm 0.8 \text{ kg/m}^2$ (21.5–37.8). Their heart rate, arterial pressure indices, CBF and CVR are shown in Table 1. CBF was positively correlated to arterial pressure indices (at least $r = 0.40$, $P < 0.05$) and inversely to MSNA ($r = -0.40$, $P < 0.05$). CVR was positively correlated ($r = 0.41$, $P < 0.03$) to MSNA.

Considering the two positions of the calf at $5.8 \pm 0.4^\circ$ or 7 ± 0.7 cm and $40 \pm 1.5^\circ$ or 41 ± 0.7 cm above horizontal, no significant changes occurred in heart rate, arterial

Table 1 Data obtained at two leg positions in the 26 subjects

Data	$5.8 \pm 0.4^\circ$	$40 \pm 1.5^\circ$	<i>P</i>
Heart rate (beats/min)	72 ± 2.3	73 ± 2.4	ns
Arterial pressure (mmHg)			
Mean	107 ± 4.2	106 ± 4.1	ns
Systolic	158 ± 5.6	157 ± 5.2	ns
Diastolic	82 ± 3.8	82 ± 3.6	ns
MSNA (bursts/100b)	59 ± 3.9	58 ± 3.7	ns
CBF ($\text{ml } 100 \text{ ml}^{-1} \text{ min}^{-1}$)	2.9 ± 0.21	3.6 ± 0.20	<0.0001
CVR (units)	41 ± 2.7	32 ± 2.0	<0.0001

Data are mean \pm SEM. *P* values refer to Student *t* tests for paired variables. CBF, calf blood flow; MSNA, muscle sympathetic nerve activity in bursts/100 cardiac beats (bursts/100b); CVR, calf vascular resistance; ns, insignificant differences.

pressure or sympathetic nerve activity (Table 1). Despite this, there was a significant increase in the plethysmographic slopes of the volume curve (from 470 ± 0.037 mV/s to 600 ± 0.036 mV/s; $P < 0.0001$), with significant increases of CBF and decreases of CVR (Table 1) amounting, respectively, to $23 \pm 4.7\%$ and $-23 \pm 3.8\%$.

In 10 of the 26 subjects, data were obtained with the calf randomly at $5.8 \pm 0.3^\circ$ or 8 ± 0.3 cm, $23 \pm 2.5^\circ$ or 13 ± 0.6 cm and $40 \pm 0.5^\circ$ or 41 ± 0.7 cm above horizontal. Again no significant changes occurred in heart rate, arterial pressure or sympathetic nerve activity (Table 2). However, compared with the lowest leg position the increase in CBF was graded according to the two raised leg positions and amounted to $13 \pm 5.9\%$ and $38 \pm 5.2\%$, respectively. Similarly there was an associated decrease in CVR amounting to $-11 \pm 4.3\%$ and $-28 \pm 6.8\%$.

Discussion

The present study has demonstrated that raising the leg above horizontal level significantly increased calf blood flow measured by venous occlusion plethysmography at constant haemodynamic variables and efferent vasoconstrictor sympathetic activity. This direct effect of leg position may constitute a confounding factor that could interfere with interventional and longitudinal assessments of calf blood flow.

The present study was designed to avoid the confounding factors of the sympathetic drive, haemodynamic variables and their reflex and direct effects on CBF. This was necessary in the design of our study that aimed to examine the direct relationship between leg position and CBF. Indeed, our previous findings⁶ and the current ones confirm that an increase in MSNA reduces CBF and increases CVR. We found that MSNA was positively correlated to CVR and inversely correlated to CBF. This occurred despite the inter-individual variations in MSNA that may be partly related to the wide range of age and body mass index in our subjects.^{19–21} In addition, in the present study, all CBF assessments were made at the same body position and when haemodynamic variables returned to baseline values; changes in body position are known to affect the efferent vasoconstrictor sympathetic drive and its reflex control.²² Changes in systemic and local arterial pressure were avoided in the present study; arterial pressure values

obtained from the upper arm were similar to those from the thigh. In the event, it was demonstrated that significant decreases and increases, respectively, of CBF and CVR occurred in response to raising the leg in the absence of changes of MSNA. Thus, the absence of significant changes in haemodynamic variables and MSNA made it unlikely that the inflow arterial pressure to the calf region could have influenced our findings. These considerations support our conclusion that the change in CBF was a direct response to the change in leg position.

As has previously been described, measurement of CBF by venous occlusion plethysmography requires supporting the leg in a position slightly above horizontal levels to avoid artifacts and to aid venous return.^{5,23} In the former study,²³ it was shown that tilting the subject to a head-down position to elevate the position of the leg relative to the horizontal level led to an increase in calf volume expansion rate, a rate that is used in measuring CBF. This increased rate of expansion was considered to reflect an increased venous emptying at baseline to an extent that allowed a faster rate of arterial inflow on venous occlusion. It was also shown that cooling to induce sympathetic activation reduced this effect.²³ Likewise, our present study also indicates that pre-emptying of veins by raising the leg leads to a greater venous capacity and subsequent increase in calf volume expansion rate upon venous occlusion. However, our design differed in that we kept the subject in the same position so as to avoid any changes in efferent vasoconstrictor sympathetic drive and in reflex control.²² Indeed, our investigation was completed in the absence of changes in vasoconstrictor MSNA that could have interfered with blood inflow and arterial blood pressure.

Our proposal that raising the leg reduces the volume of blood in the calf, thus leading to a greater venous capacity and a more rapid arterial inflow upon occluding venous return at the thigh is consistent with previous investigations using different methods.²⁴ In that study, blood volume of the calf region was measured by autologous radiolabelled erythrocytes using dynamic scintigraphy after positioning the calf in an elevated, horizontal and lowered position. Correspondingly, the calf blood volume increased 120%, 60% and 20% during venous stasis.

Our finding that elevation of the calf, and its extent, above the horizontal level can directly affect the measurement of CBF by venous occlusion plethysmography has

Table 2 Data obtained at three leg positions in the 10 subjects

Data	$5.8 \pm 0.4^\circ$	$23 \pm 2.5^\circ$	$40 \pm 0.5^\circ$	23° vs. 5.8°	40° vs. 23°
Heart rate (beats/min)	71 ± 2.9	72 ± 3.6	73 ± 3.5	ns	ns
Arterial pressure (mmHg)					
Mean	101 ± 5.5	99 ± 5.2	100 ± 5.2	ns	ns
Systolic	152 ± 5.9	150 ± 6.3	151 ± 6.8	ns	ns
Diastolic	77 ± 5.1	76 ± 4.9	76 ± 4.9	ns	ns
MSNA (bursts/100b)	60 ± 6.2	60 ± 6.0	60 ± 5.9	ns	ns
CBF (ml 100 ml ⁻¹ min ⁻¹)	2.5 ± 0.19	2.9 ± 0.25	3.6 ± 0.25	<0.05	<0.001
CVR (units)	41 ± 3.2	36 ± 3.1	29 ± 2.5	<0.05	<0.01

Data are mean \pm SEM. *P* values refer to repeated measures ANOVA post-tests, and significant differences are shown in the right two columns. CBF, calf blood flow; MSNA, muscle sympathetic nerve activity in bursts/100 cardiac beats (bursts/100b); CVR, calf vascular resistance; ns, insignificant differences.

important implications. The response of CBF to calf elevation may constitute a confounding factor in the serial measurement of calf blood flow as used in interventional and longitudinal studies. This adds to other known factors that can affect CBF, such as changes in body position, different placement of cuff or strain gauge and its size, and artifacts during acquisition of recordings.^{3–5,9,10}

In conclusion, it was demonstrated that the height of leg position above the horizontal level can directly affect the measurement of CBF by strain gauge venous occlusion plethysmography. CBF significantly increased as the calf region was elevated in steps above the horizontal level. It is suggested that this positional factor can confound the measurement of calf blood flow in longitudinal and interventional studies.

Disclosures

The authors confirm that there is no conflict of interest to disclose.

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