Posturally induced leg vasoconstrictive responses: relationship to standing duration, impedance and volume changes

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Summary

Shifting legs to a gravity-dependent position provokes a physiological vasoconstrictive response that forms the basis of several diagnostic tests based on initial (< 5 min) blood perfusion decreases. However, it is not known if responses are maintained over longer duration and if they depend on the volume shifted to the limb during the manoeuvre. These issues were investigated by measurements of blood perfusion changes on foot and ankle (laser Doppler) and below-knee volume and impedance changes induced by 30 min of standing in 10 healthy volunteers. Initial perfusion decreases were $66.4\% \pm 2.6\%$ and $49.3\% \pm 3.8\%$ for ankle and foot dorsum, respectively, and were fully maintained during sustained standing without evidence of 'vasodilator escape'. Response magnitudes were not dependent on leg volume changes using geometric or impedance measures. A close correlation ($r^2 = 0.78$) between impedance and volume changes suggests the former as a useful way of assessing dynamic limb volume changes. Sustained vasoconstrictive responses make it unlikely that extending the duration of such tests would offer more diagnostic information than is currently available.

Keywords: bioimpedance, laser Doppler, microcirculation, skin blood flow, peripheral vasculature, vasoconstriction.

Introduction

The fact that microvascular dysfunction is an important component of a several cardiovascular disease processes and complications has led to intensified efforts to develop diagnostic and mechanistic tests at the clinical level. An often-used approach is based on provocations that induce changes in skin blood perfusion that are registered by laser Doppler methods (Allen et al., 1992; Mayrovitz & Regan, 1993; Mayrovitz, 1994; Mayrovitz & Larsen, 1996a). Provocations are chosen to elicit vasodilatory (Walmsley & Goodfield, 1990; Stevens et al., 1991; Algotsson et al., 1995; Kurvers et al., 1995) or vasoconstrictive (Ekenvall et al., 1988; Coffman, 1994) responses that may be reflexly or directly induced. Depending on the specific provocation, measured responses are used to make statements about microcirculatory functional status as well as related physiological control processes and mechanism, (Algotsson, 1996; Kellogg et al., 1990, 1993) and therapeutic mechanisms and outcomes (Warren & Loi, 1995; Houben et al., 1996; Stricker et al., 1996; Wenzel et al., 1996). Postural provocations use gravity as a non-invasive way of inducing changes in intravascular blood volume and pressure to invoke vasoactive responses (Rendell et al., 1992). Shifting the lower extremities to a dependent position stresses, and thereby tests, the ability of the distal vasculature to compensate and respond to increased lower extremity blood volume and pressure. Normal responses are characterized by significant and rapid decreases in

measured blood perfusion, thought to be due to arteriolar vasoconstriction induced mainly by local neurogenic reflexes (Gaskell & Burton, 1953) with smaller contributions of local myogenic and central effects (Hassan & Tooke, 1988a). It has been argued that this response buffers the capillary and venular networks from injurious effects of increased pressure load. Abnormalities in the magnitude of the vasoconstrictive response have been shown to be associated with long-standing diabetes (Rayman et al., 1986; Belcaro & Nicolaides, 1991) as well as in young diabetic individuals (Shore et al., 1994) and in peripheral vascular diseases (Belcaro et al., 1989; Caspary et al., 1996; Mayrovitz, 1994).

In spite of its current use, there are several questions regarding the features and mechanisms underlying the vasoconstrictive response. First is the question of response duration. Most, if not all, data obtained using postural provocations are reported based on short durations not exceeding 5 min. It is not known if this response is maintained over longer durations or if adaptive recovery occurs. This is of interest for several reasons. From the physiological perspective, knowledge of the temporal behaviour of the response will further characterize its features. From the applied clinical perspective there are two issues of interest. One relates to the possibility of additional diagnostic information that might be gained from a longer test interval. The other issue relates to the potential role of this response as a preventative buffer against venous disease in individuals whose occupations require long durations of relatively motionless standing. Aside from questions of duration, there is also the question of how sensitive the vasoconstrictive response is to the amount of blood volume shifted to the dependent limb during the manoeuvre. The purpose of this study was to clarify these issues by using laser Doppler to assess the vasoconstrictive response to sustained (30 min) standing and to simultaneously assess below-knee volume changes by geometric and bioimpedance measures.

Methods

Subjects

Ten volunteer subjects (age 24–52 years, six women) participated after signing an institutional review board-approved informed consent. On the day before the experimental protocol, the normality of each subject's lower extremity arterial status was assessed with non-invasive tests, including ultrasonic Doppler measurements of ankle–brachial indices and pulsatile blood flow via nuclear magnetic resonance flowmetry (Mayrovitz & Larsen, 1996b). All subjects were well within normal ranges as judged by these vascular tests. No subject had diabetes, history of lower extremity venous disease or other neurological or cardiovascular disease. On the day of the main experimental procedures, no subject had taken any vasoactive medication and had also refrained from ingesting any vascular stimulants or depressants for at least 6 h before testing.

Below-knee leg bioimpedance

On entry into a temperature-controlled testing laboratory, subjects took a supine position on an examination table. Two current-injecting and two voltage-sensing silver–silver chloride strip electrodes (1·5 × 8 cm, Xitron) were placed at standardized sites on the anterior part of one limb. One current-injecting electrode was placed on the thigh (5 cm proximal to the knee) and the other on the distal third of the foot dorsum. Voltage-sensing electrodes were placed 2 cm distal to the knee and 2 cm proximal to the malleolus. Leads were attached to each electrode and connected to a multifrequency bioimpedance spectrometer (Xitron, model 4000B), the output of which was the impedance (real R, and imaginary X components) between the two sensing electrodes at preprogrammed frequencies of 5, 25, 50, 125, 250 and 500 kHz. Before use on each subject, the system calibration was tested and verified using a reference standard supplied with the instrumentation. Measurements on subjects were performed in triplicate at each frequency and then averaged. Differences between consecutive readings were less than 1%. The impedance magnitude (Z) was determined as the square root of $(R^2 + X^2)$. The main quantity of interest in this study was the change in Z at 5 kHz as at this frequency the impedance changes are primarily related to changes in extracellular fluid volume.

Laser Doppler

Two laser Doppler probes (Moor, P7 large area probes, Instruments, UK) were used. One was placed

on the medial foot dorsum (near the junction of toes 1 and 2) and the other on the medial gaiter about 4 cm proximal to the medial malleolus. Probes were secured with double-sided tape with the dorsum probe positioned about 2 cm distal to the furthest edge of the dorsum current-injecting electrode. The probes were connected to a dual channel laser Doppler system (Moor Instruments, MBF3D, Instruments, UK), the data from which were acquired and processed via a computer.

Below-knee leg volume

Leg circumferences were measured at six sites, starting at the proximal voltage-sensing electrode and repeated at intervals ending with the measurement of the circumference at the distal-sensing electrode. The leg volume corresponding to the region in which the bioimpedance was being determined was estimated by calculating the volume (V_{seg}) of each of the five leg segments using a frustum model according to the formula $V_{seg} = (L/12\pi) \times (C_a^2 + C_a C_b + C_b^2)$, in which C_a and C_b are the upper and lower circumferences of each segment and L is the mean distance between them. The sum of the five segment volumes was then taken as the total volume. This method for estimating lower limb volume has been shown to be essentially interchangeable with water displacement measurements (Sukul *et al.*, 1993).

Protocol

After securing probes and electrodes and making limb circumferential measurements, subjects rested for 10 min. Thereafter, during a 10-min baseline interval, laser Doppler blood perfusion was continuously monitored and recorded. At the start, middle and end of this interval; bioimpedance and volumes were also measured. The subject was then assisted to a standing position by gentle sliding to the end of the examination table. To minimize motion and muscle fatigue during standing, the buttocks were supported by the examination table edge with additional support supplied by a bar-stand that the subject used for balance. The subject remained standing for 30 min. During this standing interval, laser Doppler blood perfusion was measured continuously, bioimpedance was measured every min for the first 5 min and both

impedance and leg circumference measurements were made at 5-min intervals. At the end of the standing interval, the subject was assisted back to a supine position and remained resting for 15 min. During this supine recovery interval, laser Doppler was continuously monitored and bioimpedance and circumference measurements made at 5-min intervals starting 5 min after return to the supine position.

Results

Laser Doppler response

A typical laser Doppler perfusion response to posture shifts as recorded simultaneously at the dorsum and gaiter sites is illustrated for a single subject in Fig. 1. Transitional movements are accompanied by movement artefacts as noted in the Fig. 1. The 10-min baseline supine perfusion, which here shows some spontaneous perfusion variations, is significantly reduced on standing and is maintained at the decreased level for the 30-min standing interval. Minimal perfusion levels are achieved after about 5-min of standing. On return to the supine position (at the 40-min marker), perfusion rapidly returns to near baseline levels. Analyses of the responses of all subjects is summarized in Fig. 2. Two features of the overall response are evident: (1) the vasoconstrictive response is greater at the gaiter than at the foot dorsum; and (2) the early (5 min) and late (30 min) standing responses are essentially the same indicating sustained vasoconstriction.

Leg volume and bioimpedance changes

A typical volume response to posture shifts for a single subject is shown in Fig. 3. The shift from supine to standing was accompanied by an initial volume increase followed by a more gradual increase during the standing interval. Shifting from standing back to the supine position was associated with a return to baseline level after 15 min. A typical impedance response to posture shifts for all frequencies is illustrated for a single subject in Fig. 4. The shift from supine to standing was accompanied by an initial impedance decrease followed by a more gradual but continuous decrease during the remaining standing interval. Shifting from standing back to the
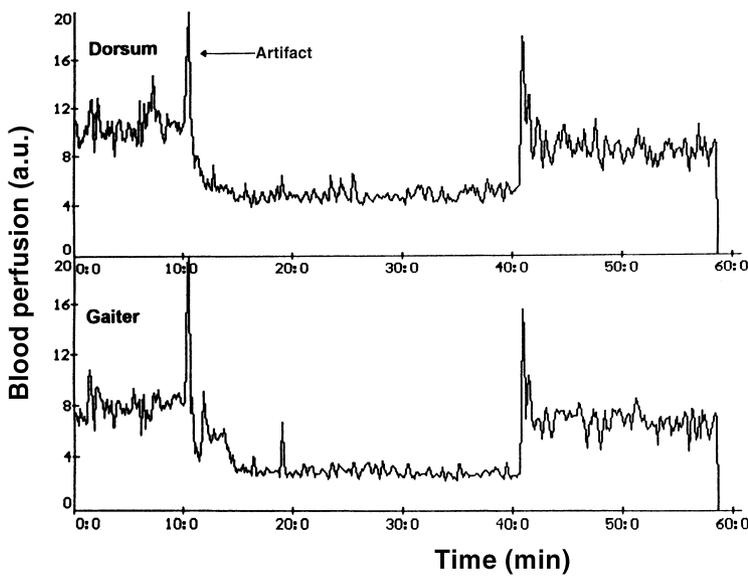
Figure 1 Typical laser Doppler perfusion response to postural shifts. Movement from supine to standing (at 10-min mark) is associated with a decrease in blood perfusion which reaches a minimum after about 5 min and remains decreased for the 30-min standing interval. Return to the supine position (at 40-min mark) results in a rapid perfusion recovery.
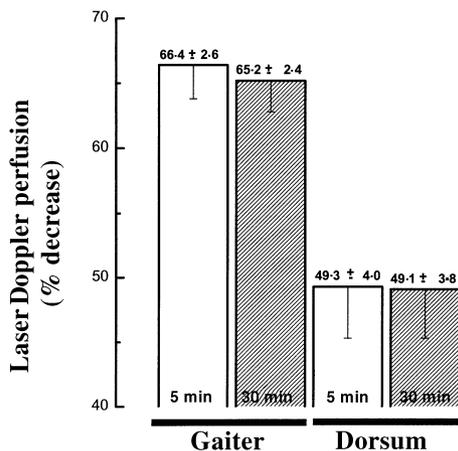

Figure 2 Early and late standing interval perfusion decreases. Percentage reductions in supine perfusion levels are greater at the gaiter than at the dorsum ($P < 0.05$) but early (5 min) and late (30 min) values do not differ at either site.

supine position was associated with a gradual return to baseline levels after 15 min. This basic response was noted at all frequencies tested. A comparison of the temporal relationship between relative impedance changes at 5 kHz and leg volume changes for all subjects is shown in Fig. 5.

Under supine resting conditions below-knee absolute impedance at 5 kHz was $133.5 \pm 6.5\Omega$ (mean ± SEM). Standing was associated with an impedance decrease in all subjects, which after 5 min of standing

was $122.5 \pm 6.0\Omega$ and $117.4 \pm 5.6\Omega$ after 30 min of standing ($P < 0.05$). In all subjects, the 30-min impedance value was less than the 5-min value. These absolute impedance changes correspond to decreases from baseline of $8.3\% \pm 0.7\%$ and $11.9\% \pm 0.9\%$ respectively. A highly significant inverse relationship between relative impedance and volume changes was found, as shown for all individual paired impedance volume data in Fig. 6. However, there was no correlation between the magnitude of the vasoconstrictive response and either volume or impedance changes (data not shown).

Discussion

Methodological considerations

Laser Doppler blood perfusion measurements as here used register the net red blood cell flux in a localized area of about 1 mm^2 to a depth of between 0.4 and 1.0 mm (Jakobsson & Nilson, 1993). Although adequate for following dynamic blood perfusion changes, the perfusion sample with this method is spacially restricted. Measurements of skin blood perfusion using laser Doppler imaging, which is capable of scanning large skin areas clearly, has shown that resting perfusions are heterogeneous (Wardell *et al.*, 1994). Thus, the baseline perfusion levels herein reported must be viewed as point samples with a
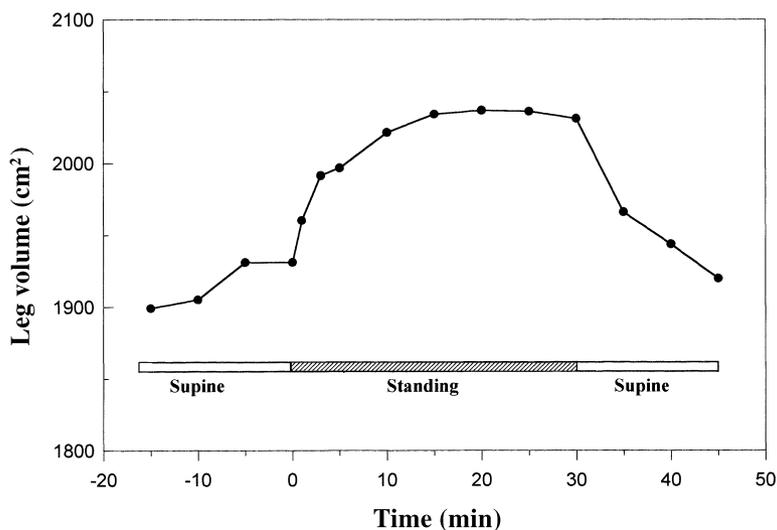
Figure 3 Typical time course of below-knee volume changes. Shifting from supine to standing causes an initial step volume increase followed by a more gradual increase during the standing interval.
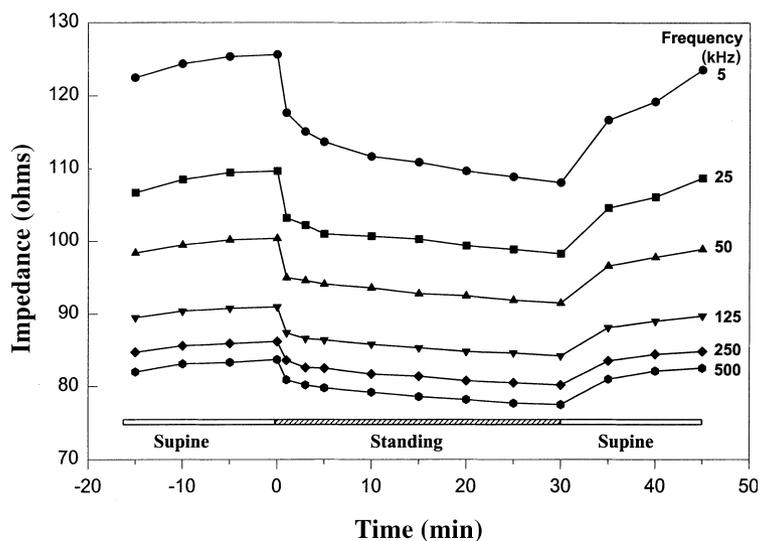

Figure 4 Impedance response to postural shifts for one subject. Shifting from supine to standing causes an initial step impedance decrease followed by a more gradual but continuous decrease during the remaining standing interval at all frequencies. At 15 min after returning to supine, impedance is at or near baseline.

possible variance at different sites as large as a factor of two. However, the relative temporal changes at a given site induced by the postural manoeuvre reflect the vasoconstrictive component within the sampled region.

Electrical impedance measurements of the lower limb as here used reflect the net impedance of all tissues and fluids within the volume between the two voltage-sensing electrodes. Shifting from a supine to a standing position is associated with increases in intravasular cellular and fluid volume and after time an increase in extravascular fluid volume. The use of multifrequency analysis should in principle allow for separation between cellular vs. fluid shifts because of frequency selectivity. At low frequencies (e.g. 5 kHz) the impedance of cellular membranes is sufficiently large compared with free fluid to interpret the low-frequency impedance changes as primarily reflecting fluid volume changes (Kanai et al., 1987).

Vasoconstrictive response

Gaskell and Burton (1953) were among the first to suggest that the initial postural vasoconstriction in
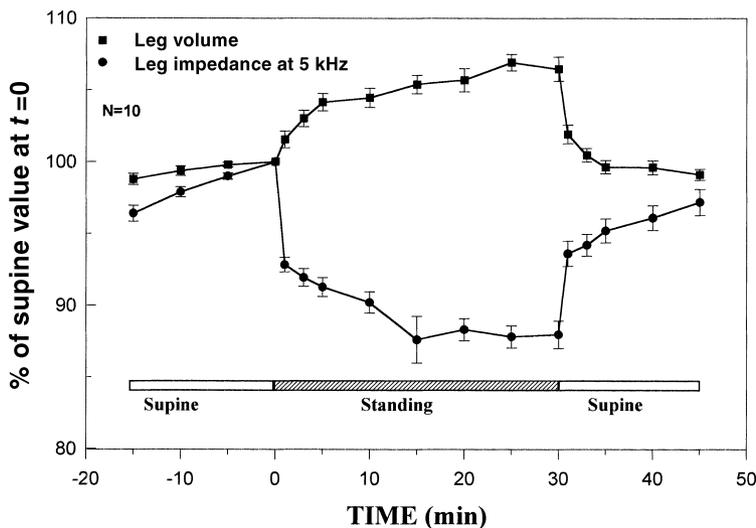
Figure 5 Overall volume and impedance temporal responses. Changes in volume and impedance are shown in terms of their percentage of the last baseline value at time = 0.
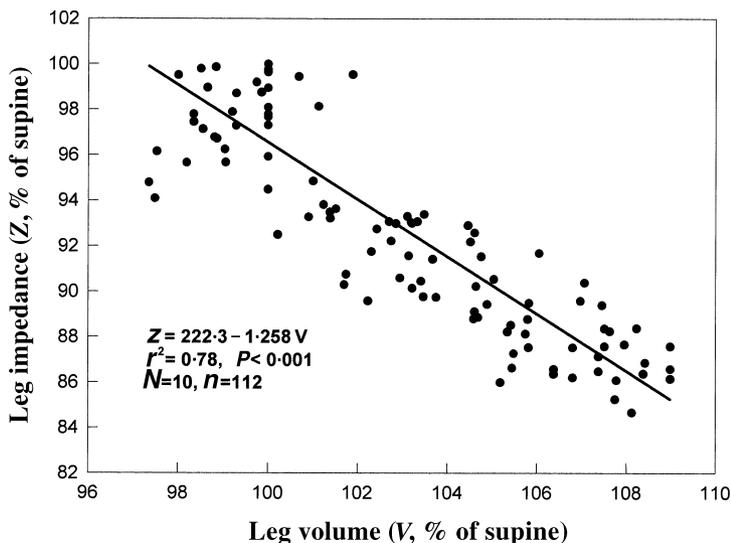

$$Z = 222 \cdot 3 - 1 \cdot 258\,V$$
$$r^2 = 0 \cdot 78, \quad P < 0 \cdot 001$$
$$N = 10, \, n = 112$$

Figure 6 Volume–impedance relationship. Individual data points ($n = 112$) are all paired volume–impedance measurements on 10 subjects. A highly significant inverse correlation between impedance and volume changes is noted. The solid line is the linear regression line whose equation and statistics are shown.

limbs was due to a local veno-arteriolar reflex. In a series of elegant studies, Hassan and Tooke (1988b) established that the response was indeed mainly due to a local neurogenic mechanism with some myogenic and small central components. Further clarification of operant mechanisms have recently been reported (Jepsen & Gaehtgens, 1995). The fact that in the present experiments the response remained uniform and intact for the entire standing interval clearly demonstrates that the primary vasoconstrictive stimulus was also maintained. This is consistent with a venous–arteriolar reflex pathway activated by venous distension that, under the present experimental conditions, would have remained distended during the standing interval. However, it has previously been noted that the vasoconstrictive response is dependent on both environmental and local temperatures, with elevations above critical physiological ranges associated with significantly reduced vasoconstrictive responses (Hassan & Tooke, 1988a). Reductions in and losses of postural vasoconstrictive responses have also been reported in patients with significant peripheral vascular disease without heating and in normal subjects when local heating was used (Ubbink et al.

1991). These combined findings suggest a competitive process between the veno-arterial constrictive mechanism and a local arteriolar vasodilatory stimulus. Restoration of arteriolar tone in patients after lower extremity revascularization procedures correlates with the return of posturally induced vasoconstrictive responses (Ubbink et al., 1992).

The magnitude of the response depends somewhat on the anatomical site, as shown here, and also on the method of eliciting the response. In the present study, a supine–standing provocation was used for two reasons. Firstly, it produces the largest change in lower extremity hydrostatic pressure. Secondly, compared with tilt-table manoeuvres or passive limb lowering, it better mimics conditions experienced by those whose occupations necessitate long periods of relatively motionless standing, such as surgeons and laboratory technicians. Such occupations are associated with an increased risk of developing lower extremity venous pathology related to the hydrostatic load. As postural vasoconstriction helps buffer against capillary pressure increases and oedema formation, the question whether the physiological buffer system is sustained is best tested under standing conditions.

Under the present test conditions, the gaiter area was found more vasoactively responsive than foot dorsum, with mean response values of about 66% and 49% respectively. This is of considerable note as the gaiter region is the site most prone to the development of venous pathology (Bull et al., 1995). Other values reported for responses on foot dorsum in normal individuals subjected to passive leg dependency have been as high as 82% when subjects remained in the supine position (Rayman et al., 1986) and as low as 28% when subjects moved to a seated position with legs lowered (Gniadecka et al., 1994). However, under the conditions simulated in the present design, it is now possible to confirm that in healthy subjects during sustained motionless standing the vasoconstrictive buffer system remains operative.

Leg bioimpedance and volume changes

The time course of the overall impedance change demonstrates a dynamic process occurring during the standing interval that is naturally separable into at least two and possibly three components: an immediate large reduction in impedance occurring within the first 2 min, a more gradual reduction in impedance that appears to reach a nadir after 15 min of standing, and a plateau interval in which little or no further impedance change is measured. In some individuals, the plateau interval is absent and small impedance changes continue throughout the 30-min standing interval. These events closely parallel the temporal course of the measured volume changes, although in an opposite direction. The fact that the fractional change in impedance at the end of the standing interval was about twice that of the volume change (−12% vs. +6%) suggests that the impedance method is more sensitive to fluid volume shifts within the dependent limb.

In summary, the present findings show that initial vasoconstrictive responses triggered by movement from the supine-to-standing position are maintained during sustained standing without evidence of vascular adaptation or 'vasodilator escape'. The magnitude of the response is not significantly affected by the amount of leg volume change assessed, either by geometric measurements or by leg impedance changes during the postural manoeuvre. The close correspondence between the pattern of ankle-to-knee impedance change and estimated limb volume changes suggests the former as a useful way of assessing or indexing dynamic limb volume changes. However, because of the consistency of the sustained vasoconstrictive response, at least in the healthy group studied, it appears unlikely that extending the duration of such tests would offer significantly more diagnostic information than is currently available via a 5-min provocation.

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