Vasoconstriction during venous congestion: effects of the veno-arteriolar response, myogenic reflexes and hemodynamics of changing perfusion pressure

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Running head: Methodology to measure cutaneous venoarteriolar response

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ABSTRACT

The purpose of this study was to dissect the relative contribution of arteriovenous hemodynamics, the venoarteriolar response (VAR), and the myogenic reflex leading to a decrease in local blood flow induced by venous congestion. Skin blood flow (SkBF) was measured in 12 supine healthy subjects (7 males and 5 females; 35±9 years, mean±SD) via laser-Doppler flowmetry: 1) over areas of forearm and calf skin in which the VAR was blocked by using Eutectic Mixture of Local Anesthetics (EMLA, 2.5% lidocaine and 2.5% prilocaine, EMLA sites); and 2) over the contralateral forearm or calf skin (Control sites), using two different techniques: limb dependency of 23–37 cm below the heart, and cuff inflation to 40 mmHg. We found that during limb dependency SkBF decreased by 45±9% in the forearm, and by 40±20% in the calf at the Control sites (both P<0.001), whereas SkBF remained unchanged at the EMLA sites (forearm, -5±23%, P=0.973; calf, -2±32%, P=0.825). In contrast, during cuff inflation, SkBF decreased by 57±13% in the forearm, and by 54±13% in the calf at the Control sites, while it also decreased at the EMLA sites by 34±11% in the forearm, and by 37±17% in the calf (all P<0.001). The percent change in SkBF from baseline was significantly greater during cuff inflation than limb dependency at both the Control sites (forearm, P=0.003; calf, P=0.011) and the EMLA sites (forearm and calf, P<0.001). Estimated skin vascular resistance remained unchanged at the EMLA sites during cuff inflation as well as limb dependency both in the forearm and the calf. These data suggest that the decrease in SkBF during venous congestion with cuff inflation is not solely due to the cutaneous VAR, but also to a reduction in local perfusion pressure between arteries and veins. The VAR is therefore most specifically quantified by venous congestion induced by limb dependency, rather than cuff inflation. Finally, from both techniques (based on the assumption that each mechanism of the decrease in SkBF is additive),
we calculated that during venous congestion induced by limb dependency (calf), ~45% of the non-baroreflex vasoconstriction is induced by the VAR and ~55% by the myogenic reflex.

**KEY WORDS**: vasoconstriction; local circulatory control; axon reflex
INTRODUCTION

When venous pressure in a limb is elevated to pressures greater than 25 mmHg, cutaneous, subcutaneous, and muscle vascular resistances increase within that region, resulting in a reduction in blood flow of ~40% (2,5,16,17,31,32). This reflex has been termed the venoarteriolar response (VAR), since stretch receptors reported to be located in small veins are hypothesized to cause changes in arteriolar vascular tone ‘upstream’ of the veins (15). During orthostasis, it has been suggested that as much as 45% of the increase in systemic vascular tone is due to the VAR, with the remaining 55% due to central reflex mechanisms elicited via baroreceptor unloading (15,19,20). An attenuated VAR may be one possible mechanism for a decrease in orthostatic tolerance after simulated microgravity exposure (38) or in patients with the Postural Orthostatic Tachycardia Syndrome (34). In addition, it is likely that the VAR contributes to the maintenance of orthostatic tolerance in individuals with spinal cord transection (28,33,35). Thus, the VAR appears to play a critical role in human cardiovascular control during orthostatic stress, and is a key variable requiring careful quantification during examination of the effect of gravitational perturbations (10,34,35,38).

The VAR has been evaluated during venous congestion induced by lowering the arm or leg below the heart level (5,10,14,21,25,27,33,34,36), during locally applied negative pressure (32), or during proximal cuff inflation (14,15,19,24,30,34,38). However, during cuff inflation, the consequent reduction in blood flow may not be due solely to the VAR, because cuff inflation uniquely decreases local perfusion pressure between arteries and veins that may reduce blood flow in the absence of VAR-induced vasoconstriction. Surprisingly, the hemodynamic effect of cuff inflation on the assessment of the VAR has not been examined. Moreover, limb dependency may engage the myogenic reflex (6,9,22) as well as the VAR.
The purpose of this study was to dissect the relative contribution of arteriovenous hemodynamics, the venoarteriolar response (VAR), and the myogenic reflex to the vasoconstriction induced by venous congestion. To accomplish this objective, we compared changes in forearm and calf skin blood flow (SkBF) during cuff inflation and limb dependency at the sites where the VAR was blocked by local anesthesia (1,5,14,15,19,20,32,36,37) and at unblocked sites.

METHODS

Subjects

Twelve healthy subjects (7 males and 5 females; age, 35±9 years, mean±SD; height, 171±11 cm; weight, 68.0±14.9 kg) participated in this study. The study was performed in accordance with the Declaration of Helsinki and all subjects signed an informed consent form approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center and Presbyterian Hospital of Dallas.

Protocol

The topical anesthetic Eutectic Mixture of Local Anesthetics (EMLA cream, 2.5% lidocaine and 2.5% prilocaine, AstraZeneca LP, Wilmington, DE) was placed on both forearm and calf skin to block neurally mediated skin vasoconstriction, and was covered by a Tegaderm dressing at least 2 hr prior to the experiment. After that period of time, the dressing and the EMLA cream were removed. The effectiveness of the cream was assessed by testing blockade of tactile sensation from that area.
The experiment was carried out 2 to 4 hr after a light meal in a quiet, environmentally controlled laboratory with an ambient temperature of ~25°C. SkBF was measured during limb dependency and cuff inflation over areas where the VAR was blocked with EMLA cream (EMLA sites) and over areas of the contralateral forearm and calf intact skin (Control sites). After observing establishment of stable SkBF (usually 20 to 30 min supine), baseline data were collected for 2 min. Then limb dependency or cuff inflation was applied for 2 min and measurements were repeated. After a sufficient recovery period (at least 2 min, when SkBF returned to the baseline level), baseline data collection was repeated. Then, the alternative intervention (cuff inflation or limb dependency) was applied. Choice of limb (right or left) for the EMLA sites, the order of the measurement at the EMLA and Control sites, calf and forearm and the order for limb dependency or cuff inflation were randomized and counterbalanced among subjects.

Cuff inflation was set at 40 mmHg (Hokanson), which increases venous pressure to a similar magnitude (4,11,13). Since limb venous pressure in supine humans ranges from 7 to 12 mmHg (mean value of ~10 mmHg) in forearms, and from 10 to 20 mmHg (mean value of ~15 mmHg) in legs (4,26,28), the increase in venous pressure by cuff inflation is estimated to be ~20 to 30 mmHg. Because cuff inflation to 40 mmHg is lower than diastolic pressure, limb arterial pressure is not affected significantly (12), and local perfusion pressure (arterial minus venous) decreases in accordance with the increase in limb venous pressure.

Arm or leg dependency was engaged by lowering the subject’s arm or lower leg off the edge of the table such that the sites of SkBF measurement were 30±3 (range, 25–37) and 30±4 (range, 23–36) cm below the heart level, respectively to match the increase in venous pressure during cuff inflation. With limb dependency, transmural pressure in the arteries increases in
accordance with the hydrostatic pressure gradient between the heart and the limb (7,28). Acutely, venous pressure may not increase proportionally to the same hydrostatic pressure gradient because the venous valves restrict the backward flow. However, as blood continues to flow from the arteries into the dependent veins, they are filled up with blood and the venous valves forced open in a heartward progression until there is an uninterrupted hydrostatic column between the central circulation and the limbs (28). Thus, once all venous valves are open, the venous transmural pressure is the sum of the dynamic pressure and the hydrostatic pressure, the same as in the arteries (28). Therefore, the premise of this study (and the venoarteriolar response) is that there is no net change in perfusion pressure in the limb during limb dependency.

**Measurements**

SkBF was measured via laser-Doppler flowmetry (Perimed, Järfälla, Sweden). A laser-Doppler flow probe was placed within the region of anesthesia for the EMLA sites or a similar region without anesthesia on the contralateral forearm or calf for the Control sites. Local temperature was controlled at 34°C via a local heating element (Perimed). The typical error of repeated measurements of the assessment of the VAR (relative decrease in SkBF from baseline) with leg dependency in our laboratory is 14.8%. Heart rate (HR) was measured by lead II of the electrocardiogram (Hewlett-Packard) and beat-by-beat arterial blood pressure (BP) was obtained by finger photoplethysmography (Finapres, Ohmeda) with the finger placed at the heart level. SkBF, HR and BP data were recorded at a sampling frequency of 250 Hz via a commercial data acquisition system (Biopac, Santa Barbara, CA). Beat-to-beat values of HR, systolic (SBP), and diastolic BP (DBP) were obtained by using a custom program for peak detection. Beat-to-beat values of mean arterial pressure (MAP) were calculated as (SBP-DBP)/3+DBP. The last 20 sec
of data for each 2 min of measurement were averaged and used for analysis. Also, arm BP was measured before each baseline measurement by electrosphygmomanometry (Suntech), with a microphone placed over the brachial artery to detect Korotkoff sounds.

**Estimation of skin vascular resistance**

To determine if a decrease in SkBF during cuff inflation and limb dependency was associated with local vasoconstriction, an index of skin vascular resistance (SkVR) was estimated from [(arterial pressure, Pa)-(venous pressure, Pv)]/SkBF (11). At baseline, Pa was assumed equal to MAP obtained by finger photoplethysmography, and Pv was assumed to be equal to 10 (range from 7 to 12 mmHg) in the forearm and 15 mmHg (range from 10 to 20 mmHg) in the leg (4,26,28). During cuff inflation, Pa was equal to MAP and Pv was 40 mmHg (inflation pressure) (4,11,13,34). During limb dependency, Pa and Pv were assumed to increase similarly depending on the distance between the measuring site and the heart level (18,28).

**Data analysis and statistics**

Data were expressed as mean±SD for 12 subjects. SkBF was normalized to the baseline value and is presented as a percent change from baseline (%ΔSkBF) (5). The effects of local anesthesia, limb dependency, and cuff inflation on measured variables were determined by using two-way ANOVA with repeated measures. Subsequent post hoc tests to determine significant differences in the various pairwise comparisons were performed by using Bonferroni corrected t-tests. All statistical analysis was performed with a personal computer-based analysis system (SigmaStat 3.00, SPSS).
RESULTS

HR and MAP did not change during limb dependency or cuff inflation (Table 1). Original tracings of SkBF from a representative subject during cuff inflation and limb (leg) dependency at the Control site and the EMLA site are shown in Figure 1. Forearm and calf SkBF during cuff inflation and limb dependency are shown in Table 1, and %ΔSkBF in the forearm and the calf are presented in Figure 2. There was no significant difference in baseline forearm and calf SkBF between the Control and the EMLA sites both prior to limb dependency (forearm, P=0.195; leg, P=0.208) and cuff inflation (forearm, P=0.464; leg, P=0.094).

During limb dependency, SkBF at the Control sites decreased by 45±9% in the forearm and by 40±20% in the calf (both P<0.001), whereas it remained unchanged at the EMLA sites (forearm, -5±23%, P=0.973; calf, -2±32%, P=0.825). %ΔSkBF during limb dependency was significantly smaller at the EMLA sites than the Control sites both in the forearm and the calf (both P<0.001). On the other hand, during cuff inflation, SkBF at the Control sites decreased by 57±13% in the forearm and by 54±13% in the calf (both P<0.001). SkBF also decreased by 34±11% in the forearm, and by 37±17% in the calf at the EMLA sites (both P<0.001), although %ΔSkBF during cuff inflation remained significantly smaller at the EMLA sites than the Control sites both in the forearm and the calf (both P<0.001). %ΔSkBF was significantly greater during cuff inflation than limb dependency both at the Control sites (forearm, P=0.003; calf, P=0.011) or at the EMLA sites (forearm and calf, P<0.001).

SkVR index in the forearm and the calf during cuff inflation and limb dependency are shown in Table 1. SkVR remained unchanged from baseline at the EMLA sites during cuff inflation (forearm, -3±21%, P=0.986; calf, 10±32%, P=0.218) as well as limb dependency
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(forearm, 12±21%, P=0.055; calf, 14±36%, P=0.188). SkVR increased at the Control sites both during cuff inflation (forearm, 57±58%, P=0.004; calf, 52±57%, P=0.011) and limb dependency (forearm, 90±27%; calf, 87±67%; both P<0.001), suggesting that the reduction in SkBF during cuff inflation at the EMLA sites was not associated with the VAR but with the decrease in perfusion pressure.

**DISCUSSION**

The major findings of the present study are that: 1) forearm and calf SkBF at the VAR blocked sites remained unchanged during limb dependency, but decreased during cuff inflation; 2) decreases in forearm and calf SkBF were significantly greater during cuff inflation than during limb dependency both at the control sites and the blocked sites; and 3) forearm and calf SkVR at the VAR blocked sites remained unchanged during cuff inflation as well as limb dependency.

**The VAR during limb dependency**

The VAR is mediated through a local mechanism (1,5,8,37), and the central nervous system is not necessary to evoke the response (20,25). This construct is supported by the observations that the VAR persists during acute spinal and sympathetic neural blockade proximal to the site of measurement (14,15,19,20,31,36), in denervated skin flaps (39), and in areas distal to the lesion in spinal cord transection patients (2,33,35). In the present study, subjects remained otherwise supine and there were no changes in HR or arterial pressure during each test; thus changes in SkBF during each method were not related to central baroreflex mechanisms.

During limb dependency, an increase in hydrostatic pressure (28) causes an increase in intravascular pressure within that region (7). Increased venous pressure, especially in the venula
has been shown to be related to the magnitude of vasoconstriction within that region produced by the VAR. The increased venous pressure is thought to activate stretch receptors located in small veins and to cause vasoconstriction via a sympathetic axon reflex. Crandall et al. (5) found that the response is mediated by non-adrenergic, but neurally mediated local mechanisms by demonstrating that cutaneous vasoconstriction during arm dependency was preserved in areas of skin treated with selective and nonselective $\alpha$-adrenergic antagonists, as well as in areas of skin treated with bretylium tosylate, which blocks neurotransmitter release from adrenergic nerves (23).

The arterial myogenic response related to increases in arterial pressure may also contribute to the changes in systemic vascular resistance during orthostasis (21,25,27,39) and to vasoconstriction during limb dependency (6,9,22). Although the exact signal transduction pathways underlining the myogenic response remain uncertain, it has been thought that vascular smooth muscle depolarization culminates in increased intracellular $Ca^{2+}$ levels with mechanically induced calcium entry via voltage gated calcium channels and subsequent smooth muscle vasoconstriction (6,22). However, one recent study by Scotland et al. (29) suggested in the rat mesenteric arteries that elevation of intraluminal pressure is associated with generation of arachidonate metabolites, that in turn, activates vanilloid receptors TRPV1 on C-fiber nerve endings, resulting in depolarization of nerves and consequent release of vasoactive sensory neuropeptides. This pathway then causes constriction of vascular smooth muscle by binding to tachykinin NK1 receptors. Regardless of the specific pathway and consistent with prior studies (5,36), the skin vasoconstriction was completely blocked during limb dependency at the EMLA sites in the present study. EMLA induced inhibition of vasoconstriction with limb dependency is not due to impaired vasoconstriction of vascular smooth muscle, but rather due to inhibiting local
neural communication required for the local vasoconstriction (14,15,19,36), because lidocaine treatment does not impair cutaneous vasoconstriction to local application of noradrenaline (5). Therefore, if the myogenic response contributes to vasoconstriction during limb dependency, the result of the present study also supports the idea that vascular sensory fibers are involved in mediating the myogenic response (29).

Changes in SkBF during cuff inflation

Cuff inflation at a pressure lower than diastolic pressure impedes venous return, resulting in venous congestion and subsequent increases in venous pressure equivalent to the inflated cuff pressure (4,11). However, in contrast to limb dependency, this maneuver minimally affects the local arterial circulation (12) and thus the arterial myogenic response is not likely to be activated during cuff inflation (9,21,24,26,34). Therefore, this maneuver has been used to estimate the VAR independent of the arterial myogenic response (21,24,34,38).

In the present study, we showed that forearm and calf SkBF at the EMLA sites decreased significantly during cuff inflation. In addition, %ΔSkBF in the forearm and the calf was significantly greater during cuff inflation than limb dependency both at the Control sites and the EMLA sites, despite the similar increases in venous pressure during each method. These results strongly indicate that the decrease in SkBF during cuff inflation is not solely related to the cutaneous VAR.

During venous congestion with cuff inflation, local perfusion pressure between arteries and veins decreases because venous pressure increases up to the cuff pressure (4,11,13) without changes in arterial pressure (12). In the present study, we found that the SkVR estimated from SkBF and local arterial and venous pressure remained unchanged at the EMLA sites during cuff
inflation. This finding suggests that the observed decrease in SkBF during cuff inflation at the EMLA sites is not caused by the VAR, but rather by the decrease in local perfusion pressure. Based on this observation we would assume that a similar portion of the decrease in SkBF during cuff inflation at the Control sites is also caused by the same hemodynamic effect.

In contrast to the present findings, Henriksen and Sejrsen (19) reported that there was no reduction in skin blood flow at lidocaine treated sites during cuff inflation of 40 mmHg. One possible explanation for the discrepancy in responses between the present and the prior study could be the difference in concentration of lidocaine or the volume administered. They injected a very large dose of lidocaine (1 ml of a 20 mg/ml solution) intracutaneously and data collection began 20 min after the injection (19). It is possible that the high concentration of lidocaine could have caused pronounced vasodilation. In addition, 20 min may not be long enough to eliminate vasodilatation associated with injection trauma (3,5). The pronounced vasodilation would have opposed the reduction in perfusion pressure due to cuff inflation (5,39). In contrast, we applied lidocaine by dermal application of EMLA cream at least 2 hr prior to the experiment, and there was no significant difference in baseline forearm and calf SkBF between the Control sites and the EMLA sites in the present study.

**Implications**

Possible mechanisms of the decreases in SkBF during each test in the present study are listed in Figure 3. Based on the assumption that each mechanism of the decrease in SkBF is additive, during cuff inflation, the decreases in SkBF at the Control sites are caused by the hemodynamic changes plus the VAR, whereas those at the EMLA sites are solely due to the changes in hemodynamics. Therefore, it is reasonable to suggest that the difference in %ΔSkBF
between the control and EMLA sites during cuff inflation (Difference 1; the Control sites minus the EMLA sites; forearm, 23±15%; calf, 17±24%) may reflect the magnitude of vasoconstriction due to the VAR. In contrast, during limb dependency, the decreases in SkBF at the Control sites are associated with the VAR and the myogenic response, while we assumed that there are no such mechanisms active for the decreases in SkBF at the EMLA sites. Consequently, the difference in %ΔSkBF between the control and EMLA sites during limb dependency (Difference 2; the Control sites minus the EMLA sites; forearm, 40±27%; calf; 38±31%) may reflect the magnitude of vasoconstriction due to the VAR and the myogenic response. Furthermore, the differences between cuff inflation and limb dependency in the solutions of the above equations (Difference 3; forearm, A – B, 17±22%; calf, a – b, 21±28%) may reflect the magnitude of vasoconstriction due to the myogenic response. Ultimately then, we suggest that the percent contributions of the VAR and the myogenic response to the vasoconstriction during limb dependency are 58% (A/B × 100) and 42% (C/B × 100) in the forearm, and 45% (a/b × 100) and 55% (c/b × 100) in the calf, respectively. Therefore, it is also suggested that the vasoconstriction due to the non-baroreflex mechanisms during orthostasis may be including the effect of the myogenic response (21,25,27,39) by ~40 to 60%.

**Study limitations**

There are at least four limitations to the present study. First, the cutaneous VAR was compared during limb dependency and cuff inflation using laser-Doppler flowmetry. We recognize that the cutaneous vasculatures do not play an important role in blood pressure regulation during orthostasis under normothermic conditions. However, by assessing skin blood flow during the perturbation, it is possible that important information can be obtained that might
provide a clue as to what is occurring in vascular beds that are more important to blood pressure regulation during orthostasis but are less accessible. Second, we did not measure local venous and arterial pressure directly. Hence, we could not distinguish the exact portion of the decrease in SkBF during cuff inflation at the Control sites that was caused by a reduction in perfusion pressure. Third, we assumed (but cannot prove) that the increase in transmural pressure from hydrostatic pressure gradients during limb dependency was the same in the arteries and the veins. Our results that estimated SkVR did not change during limb dependency at the EMLA sites argue in favor of this assumption. It should be noted that a dissociated increase in intravascular pressure between the arteries and the veins from supine to sitting position has been reported by direct measurements in the hallux (7). However the measurements in that study were performed after at least half an hour, which was considerably different from the short (minutes) period of limb dependency in the present study and in previous reports (5,10,14,21,25,27,33,34,36). Direct measurements of transmural pressure in the arteries and the veins at the extremities during limb dependency within a few minutes are necessary to clarify this question. Fourth, we calculated the contributions of the VAR and the myogenic response to the vasoconstriction during limb dependency based on the assumption that each mechanism of the decreases in SkBF during cuff inflation and limb dependency is additive (Figure 3). However, this assumption needs to be verified in future studies.

In conclusion, a decrease in skin blood flow during venous congestion by using cuff inflation is not solely due to the cutaneous VAR, but rather is also due to changes in regional hemodynamics, with a decrease in local perfusion pressure between arteries and veins. Thus, assessment of the VAR from a decrease in local blood flow using cuff inflation overestimates the
magnitude of the response, and should therefore be performed cautiously. The effects of hemodynamics during cuff inflation may be minimized when the local vascular resistance is calculated and used to evaluate the VAR. Finally, from both techniques, we calculated that during venous congestion induced by limb dependency (in the calf), ~45% of the non-baroreflex vasoconstriction is induced by the VAR and ~55% by the myogenic reflex.
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FIGURE LEGENDS

Figure 1. Original tracings of skin blood flow from one representative subject comparing normalized skin blood flow during cuff inflation (A) and limb (leg) dependency (B) at a Control site (solid lines) and EMLA treated site (dotted lines). Data were originally sampled at 250 Hz; however, for display purpose these data were resampled at 4 Hz.

Figure 2. Percent changes in skin blood flow (A, forearm; B, calf) during cuff inflation (40 mmHg) and limb dependency (forearm, 30±3 cm; calf, 30±4 cm; mean±SD) at the EMLA treated sites (EMLA sites, closed bars) and contralateral control sites (Control sites, open bars). Means and SD bars are presented in 12 subjects.

Figure 3. Possible mechanisms of the decreases in SkBF during cuff inflation and limb dependency at the Control sites and at the EMLA sites. VAR, venoarteriolar response. The percent change in skin blood flow from baseline (%ΔSkBF) is presented. Also, the difference in %ΔSkBF between the Control and EMLA sites is calculated, which may reflect the magnitude of vasoconstriction due to the VAR during cuff inflation (Difference 1) and due to the VAR and the myogenic response during limb dependency (Difference 2). The differences between cuff inflation and limb dependency in the solution of the difference in %ΔSkBF between the Control and EMLA sites may reflect the magnitude of vasoconstriction due to the myogenic response (Difference 3).
REFERENCES


Table 1. Changes in skin blood flow, estimated skin vascular resistance, heart rate, and mean arterial pressure during cuff inflation and limb dependency at the Control and the EMLA treated sites.

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<th>Limb dependency</th>
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<td>30±10*</td>
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<td>59±28*†</td>
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<td>SkBF, AU</td>
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Values are mean±SD, n=12. SkBF, skin blood flow; SkVR, skin vascular resistance; AU, arbitrary units; HR, heart rate; MAP, mean arterial pressure. * P<0.05, compared to baseline; † P<0.05, compared to the Control sites, # P<0.05, compared to cuff inflation.
Figure 1

Panel A: Graph showing the normalized skin blood flow over time, with dashed line indicating the control site and solid line indicating the EMLA site. The graph shows a decrease in blood flow following cuff inflation.

Panel B: Graph similar to Panel A, showing the effect of dependency on normalized skin blood flow. The graph indicates a decrease in blood flow following dependency.

Figure 1
Changes in Calf Skin Blood Flow, %
P=0.011

Changes in Forearm Skin Blood Flow, %
P=0.003

Figure 2
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### Cuff inflation

**Control sites**
- 1) Hemodynamics
- 2) VAR

**EMLA sites**
- 1) Hemodynamics
  - Forearm (57±13%)
  - Calf (54±13%)
- 2) VAR
  - Forearm (34±11%)
  - Calf (37±17%)
- 2) Myogenic
  - Forearm (45±9%)
  - Calf (40±20%)

**Limb dependency**

**Control sites**

**EMLA sites**
- 1) VAR
  - Forearm (45±9%)
  - Calf (40±20%)
- 2) Myogenic
  - Forearm (5±23%)
  - Calf (2±32%)

### VAR

**Difference 1**
- Forearm (23±15%) – A
- Calf (17±24%) – a

### VAR + Myogenic

**Difference 2**
- Forearm (40±27%) – B
- Calf (38±31%) – b

### Myogenic

**Difference 3**
- Forearm (17±22%) – C
- Calf (21±28%) – c

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Figure 3