Sex-related effects on venous compliance and capillary filtration in the lower limb.

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Running head: Venous compliance, capillary filtration and sex.

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Abstract

Recent studies in humans have suggested sex differences in venous compliance of the lower limb, with lower compliance in women. Capillary fluid filtration could however be a confounder in the evaluation of venous compliance. The venous capacitance and capillary filtration response in the calf of 12 women (23.2±0.5 years) and 16 men (22.9±0.5 years) were studied during eight min lower body negative pressure (LBNP) of 11, 22 and 44 mmHg. Calf venous compliance is dependent on pressure and was determined using the first derivative of a quadratic regression equation that described the capacitance-pressure relationship (compliance = \( \beta_1 + (2*\beta_2* \text{transmural pressure}) \)). We found a lower venous compliance in women at low transmural pressures and the venous capacitance in men was increased (P < 0.05). However, the difference in compliance between sexes was reduced, and not seen at higher transmural pressures. Net capillary fluid filtration and capillary filtration coefficient (CFC) were greater in women than in men during LBNP (P < 0.05). Furthermore, calf volume increase (capacitance response + total capillary filtration) during LBNP was equivalent in both sexes. When total capillary filtration was not subtracted from the calf capacitance response in the calculation of venous compliance the sex differences disappeared, emphasizing that venous compliance measurement should be corrected for the contribution of CFC.

Keywords: LBNP, CFC, compliance, sex, venous capacitance
Introduction

The venous section of the cardiovascular system can be looked upon as a voluminous blood reservoir (70% of total blood volume), designed to preserve a proper inflow of blood into the heart during various cardiovascular adjustments. Thus, central venous pressure and filling of the heart may be maintained at a fairly stable level, despite variations in venous blood volume (49). During upright posture however, the pooling of blood in the veins of the lower part of the body decreases central blood volume and venous return (4, 5, 16). The venous compartment in the legs, rather than the pelvic or abdominal region, seems to have a hemodynamic impact during lower body negative pressure (LBNP) (16), and in studies on men a greater calf venous compliance has been linked to an increased venous capacitance response with a concomitant reduction in central blood volume (44, 56). This in turn elicits an increased sympathetic response with higher peripheral resistance and increased heart rate (4, 5, 10, 16, 44, 58). Thus, venous compliance of the lower limb may have an impact on cardiovascular responses to orthostatic stress and orthostatic tolerance although there might be differences between sexes confounding such a link (4).

Women are more susceptible to orthostatic stress than men (4, 10, 13, 40, 51, 58), and in accordance with some findings in the arterial tree, it may be hypothesised that women have greater venous compliance in the lower limbs predisposing to orthostatic intolerance (52). This seems to be refuted however by recent findings that demonstrate a lower venous compliance in women (34, 39). In these studies, compliance has been evaluated based on the calf volume – venous pressure relationship after several minutes of venous stasis although capillary fluid filtration significantly increases calf tissue volume (26). Huxley et al (2005) used a large experimental animal model and found increased micro-vessel permeability after administration of adenosine only in females (23). To the best of our knowledge, no previous
study has assessed sex differences in capillary fluid filtration and CFC in humans, even though sex hormones seem to affect capillary permeability and body fluid homeostasis (45, 53, 55).

Due to the conflicting results with lower venous compliance in the lower extremities in women despite an increased orthostatic intolerance (34, 39), we wanted to re-evaluate sex-related differences in venous compliance and capacitance. Thus, the aim of the present study was to define the capacitance response and capillary filtration in the calf of young women and men in response to defined transmural pressure gradients, as well as to calculate venous compliance and assess the impact of capillary filtration. We hypothesized that capillary filtration would be increased in women, and would impact orthostatic load and further, that capillary filtration would significantly affect assessment of venous compliance.
Methods

A total of 28 volunteers, 12 women and 16 men (22.9±0.5 and 23.2±0.5 years), were included in the study. All subjects were healthy, with no history of cardiovascular disease and of average physical fitness. Physical examination showed absence of deep or superficial varicose veins, hypertension, diabetes or any other serious systemic disease. All were non-smokers. No subjects were taking any regular medication, except oral contraceptive pills. Each woman was scheduled in the middle two weeks of her menstrual cycle not excluding oral contraceptive use (six women), since the effect of menstrual cycle and oral contraceptives on venous compliance and LBNP response seem to be minor (8, 34, 35). Each subject gave informed consent to the experiments approved by the Ethics Committee of Linköping University, Sweden.

The experiments were performed at a stable room temperature of 23-25 °C, and started one hour after a regular meal in the morning or at noon, with half of the subjects examined randomly in the morning or in the afternoon. The subjects were instructed to abstain from coffee, tea or caffeine on the day of the investigation. Throughout the experiments that were performed at two occasions, each lasting 2-3 hours, continuous efforts were made to maintain a relaxed and quiet atmosphere.

The subjects were placed in the supine position with the legs enclosed in an air-tight box up to the level of the iliac crest with a seal fitted hermetically around the waist. The box was connected to a vacuum source (lower body negative pressure, LBNP), permitting stable negative pressure to be rapidly produced (within 5 seconds). The pressure in the LBNP chamber was continuous measured by a manometer (DT-XX disposable transducer, Viggo spectramed, Helsingborg, Sweden) and held constant by a rheostat. During LBNP, 80 % of
the negative pressure is transmitted to the underlying muscle tissue of the leg irrespective of muscle depth, time and magnitude, leading to a defined increase in transmural pressure over the vessel wall, with a concomitant vessel dilatation and blood pooling (43). Since the compliance of the arterial bed is only \( \approx 3\% \) of that of the venous bed, almost exclusively venous blood is pooled (48).

To define the hypovolemic stimulus caused by LBNP, and to measure the change in calf volume, the pooling of blood (capacitance response) in the legs and net capillary fluid filtration was measured with mercury-in-silastic strain-gauge plethysmography. This method is designed for measuring volume changes (ml 100ml\(^{-1}\)) of a limb by measuring the circumference. The strain gauge was applied at the maximal circumference of the right calf. The basal venous pressure was not measured, but care was taken to place the calf 5 cm below heart level in all subjects and to avoid any confounding external pressure, the lowest part of the calf being at least 2 cm above the floor of the LBNP chamber. Further, the subjects rested in the supine position for at least 30 min to ensure stable calf volume and arterial inflow prior to the LBNP stimulus. LBNP was then rapidly instituted and maintained for 8 min. The experiments were performed in random order at LBNP 11, 22 and 44 mmHg with at least 30 minutes in between each investigation to assure that the basal state was restored. The pressure interval used was defined according to the following considerations: Volume registrations from the calf at LBNP pressures lower than 10 mmHg may be somewhat unreliable. We therefore used 11 mmHg in the LBNP chamber as our lower limit. Since females tend to develop signs of presyncope at approximately LBNP 50 mmHg we used LBNP 44 mmHg as the upper limit for the applied negative pressure (4). After correcting for pressure transmission, the studied transmural pressure interval was 9 to 36 mmHg. This low end of the pressure-volume curve might be a more sensitive marker for differences in compliance.
according to earlier studies from our laboratory, and potential differences in venous compliance between groups of subjects may thus be easier to detect (44).

At onset, LBNP evoked an initial rapid increase of calf volume (capacitance response) followed by a slower, but continuous rise caused by net transcapillary fluid filtration from blood to tissue (Fig. 1). At cessation there was a rapid decrease of the volume corresponding with the increase at onset of LBNP (26, 43). Lundvall et al (1993) measured changes in calf volume simultaneous with measurement of technetium marked erythrocytes during LBNP, and found that the capacitance response was completed within three minutes after the institution of LBNP (32). They concluded that the volume increase after this time was due to net capillary fluid filtration in accordance with findings by Schnitzer et al (50). Thus, the capacitance response ($V_{cap}$) was calculated from the volume increase at onset of LBNP to the line defined from the filtration slope between 3 and 8 minutes (32, 50). The total capillary filtration ($V_{filtr}$) during LBNP was calculated from the rate of filtration (ml/min) times the time of the LBNP stimulus (8 min). The total calf volume increase ($V_{tot}$) was calculated as $V_{tot} = V_{cap} + V_{filtr}$. The reproducibility in measurements was good (CV 7%). Rather than measuring the calf volume at a large variety of transmural pressures we chose to obtain at least two readings at each of the three pressure levels in every individual, and the mean value taken as the prevailing capacitance response and capillary fluid filtration.

Calf venous compliance ($C$, ml 100ml$^{-1}$ mmHg$^{-1}$), was measured by a modified version of the technique developed by Olsen and Lanne (1998) (43). In short, each capacitance response was related to the increase in transmural pressure (80% of the applied negative pressure). The resulting capacitance-pressure curve was non-linear, with larger volume changes (greater compliance) at lower transmural pressures as described by a quadratic regression equation:
Calf volume = $\beta_0 + \beta_1 \times (\text{transmural pressure}) + \beta_2 \times (\text{transmural pressure})^2$  \hspace{1cm} (1)

where $\beta_0$ is the y-intercept, and $\beta_1$ and $\beta_2$ are characteristics of the slope of the volume-pressure curve. This equation showed an excellent fit to the measured data points (Fig. 2 and 6). Since the compliance is dependent on the pressure, no single value can characterise the slope of this relation. To simplify data presentation, the first derivative of the volume-pressure curve ($C = \beta_1 + 2 \times \beta_2 \times (\text{transmural pressure})$) was calculated, creating a linear compliance-pressure curve. The slope of this curve equals the derivative of the compliance-pressure curve (Slope = $2 \times \beta_2$) and was used as well as the two components $\beta_1$ and $\beta_2$ to determine differences in calf venous compliance. The impact of capillary fluid filtration when calculating compliance was studied with use of the calf volume increase caused by $V_{\text{cap}}$, i.e. without filtration, defining $C_{\text{cap}}$, which was then compared with compliance calculations where the total calf volume increase ($V_{\text{tot}}$) caused by $V_{\text{cap}} + V_{\text{filtr}}$, defining $C_{\text{tot}}$, was used.

The capillary filtration coefficient (CFC, ml 100ml$^{-1}$ min$^{-1}$ mmHg$^{-1}$) in the calf was calculated as $[CFC = \Delta V / \Delta P \times t]$, where $\Delta V$ denotes the capillary filtration during LBNP (ml 100ml$^{-1}$), $\Delta P$ denotes the LBNP induced change in transmural capillary pressure (mmHg), and $t$ denotes time (min).

All data are given with reference to soft tissue weight excluding bone with bone taken as 10% in the calf (15). Values are expressed as means ± SE. The significance of difference between the two groups was tested by unpaired student’s $t$-test and ANOVA test. Paired student’s $t$-test was used to test the difference within each group. When calculating the compliance, every subject’s own volume-pressure curve was adjusted to a regression equation, and $\beta_0$, $\beta_1$ and $\beta_2$
stored individually. Each parameter was then compared between the groups with student’s t-test. Statistical significance was set to P < 0.05.
Results

Table 1 shows the demographic values in the women and men at rest. There was no difference in age between the groups. The women were shorter than the men (P < 0.0001), weighted less (P < 0.01) with no difference in Body Mass Index. Further, women had lower systolic blood pressure (P < 0.001) and pulse pressure (P < 0.001). All subjects tolerated LBNP well, without objective or subjective signs of a vagal reaction.

Figure 2 A shows the capacitance-pressure curve created from the increase in venous capacitance response (V_cap) with increasing LBNP. V_cap during LBNP 11, 22 and 44 mmHg was 0.73±0.06, 1.34±0.11 and 2.24±0.14 (ml 100ml⁻¹) in women, and 0.91±0.08, 1.73±0.12 and 2.66±0.16 (ml 100ml⁻¹) in men. V_cap was higher in men at LBNP 22 mmHg and overall (P < 0.05). Figure 2B shows the corresponding venous compliance (C_cap) curves. It is obvious that C_cap is dependent on transmural pressure (higher at low transmural pressure), and that C_cap is significantly higher in men than in women at low transmural pressures. The C_cap curves cross at approximately 28 mmHg, and at higher transmural pressures, women seem to have greater C_cap without reaching significant difference in the pressure interval up to 36 mmHg.

Table 2 shows the parameters of the quadratic regression equation defining venous compliance in women and men. β₁ was lower and β₂ closer to zero in women than in men (P < 0.05). Further, the slope of the C_cap-pressure curve was less steep in women (–0.0013±0.00072) than in men (-0.0029±0.00034), (P < 0.05, Fig. 2B).

Figure 3 shows the capillary fluid filtration in the calf during eight min LBNP. The capillary filtration during LBNP 11, 22 and 44 mmHg was 0.042±0.005, 0.074±0.004, 0.159±0.013 (ml 100ml⁻¹) in women, and 0.030±0.003, 0.060±0.004, 0.138±0.008 (ml 100ml⁻¹) in men, with
women having larger capillary filtration at LBNP 11 and 22 mmHg, as well as overall (P < 0.05).

Figure 4 shows the capillary filtration coefficient (CFC) in the calf during LBNP. The CFC during LBNP 11, 22 and 44 mmHg was 0.0047±0.0005, 0.0042±0.0002 and 0.0045±0.0004 (ml 100ml⁻¹ min⁻¹ mmHg⁻¹) in women, with 0.0034±0.0003, 0.0034±0.0003 and 0.0039±0.0002 (ml 100ml⁻¹ min⁻¹ mmHg⁻¹) in men, with women having significantly greater CFC at LBNP 11 and 22 mmHg, as well as overall (P < 0.05).

Figure 5 shows the total calf volume increase (Vtot = Vcap + Vfiltr) during eight min LBNP. The Vtot during LBNP 11, 22 and 44 mmHg was 1.09±0.07, 1.98±0.13 and 3.58±0.20 (ml 100ml⁻¹) in women, and 1.16±0.09, 2.22±0.14 and 3.77±0.20 in men, with no sex differences. The Vfiltr in women was 49±8%, 46±4% and 59±6% of the Vcap during LBNP 11, 22 and 44 mmHg, and 28±3%, 29±2% and 43±3% in men, being greater in women at all LBNP levels (P < 0.01), as well as overall (P < 0.001). Vfiltr also contributed greater to the Vtot at all LBNP levels in women, (P < 0.001). Further, the relative contribution of Vfiltr to Vtot was increased in both sexes at LBNP 44 mmHg, (P < 0.05).

Figure 6 illustrates the calf volume-pressure curves using Vcap as well as Vtot, and their derived compliance-pressure curves (Ccap and Ctot) in women (A) and men (B). Figure 6A shows that when not accounting for Vfiltr in calf volume increase (i.e. Vtot), β₁ changed 27% (P < 0.01), and β₂ as well as the slope of the compliance-pressure curve changed 43% in women (P < 0.05). Figure 6B shows that in men, β₁ increased 15% when not accounting for Vfiltr (P < 0.05), but the changes in β₂ as well as the slope failed to reach significance (P = 0.07).
Table 3 shows a comparison of the parameters in the quadratic regression equation defining calf venous compliance between women and men using \( V_{\text{cap}} \) as well as \( V_{\text{tot}} \), in compliance-pressure curves (\( C_{\text{cap}} \) and \( C_{\text{tot}} \)). There was a significant difference between \( C_{\text{cap}} \) and \( C_{\text{tot}} \) in both women (in \( \beta_1 \) and \( \beta_2 \)), and men (only in \( \beta_1 \)) \( (P < 0.05, \text{ figure 6}) \). The lower \( C_{\text{cap}} \) in women than in men was abolished in \( C_{\text{tot}} \), when filtration was not accounted for.
Discussion

The main findings in this study were: First, calf venous compliance ($C_{cap}$) was reduced in women compared to men at low transmural pressures, with a concomitant reduction in capacitance response during lower body negative pressure (LBNP). Second, these differences were reduced and were not seen at higher transmural pressures. Third, interstitial fluid accumulation due to capillary fluid filtration was larger in women, probably due to higher capillary filtration coefficient (CFC) than in men. Forth, calf volume increase ($V_{tot} = \text{venous capacitance response} + V_{cap} + \text{total capillary filtration} + V_{filtr}$) during LBNP was similar in women and men. Fifth, the sex-related differences in $V_{cap}$ and $C_{cap}$ were hidden if the contribution of capillary filtration was not accounted for.

Venous compliance is described as the relationship between change in venous volume and distending (transmural) pressure. At low pressures, when the slope in the volume-pressure curve is steep, the compliance in the vein is high, meaning that a large change in volume accompanies only a small change in pressure. At higher pressure the slope is less steep and compliance is lower (57). This is because the early expansion of the veins involves no actual stretch of the elastic walls, but rather acts through a change in the geometry of the veins (42, 46). Once the veins have assumed a circular cross-section, subsequent increases in their transmural pressure are opposed by the development of increased tension in the walls. The volume-pressure curve of a whole limb at rest represents the distributed properties of all veins (microvessels to large veins). Other factors besides venous properties may affect this curve, such as rigid fascia that restricts expansion, especially in the upper part of the curve, at high transmural pressure gradients. The volume-pressure relationship will also be affected by the vascular anatomy, which determines how large a fraction of the total volume that is distributed within the smallest veins as opposed to the largest ones. A complex and undefined
distribution of compliances exists between the smallest venules and the largest veins. This means that total venous compliance of the limbs depends on the size, relative number, and the wall structure of each venous segment (49).

When measuring calf volume changes during LBNP, it is of fundamental importance to be able to separate filling of the capacitance vessels from the capillary filtration. This is aided by the fact that the capacitance response is a rapid process terminated within approx 3 min, whereas the capillary filtration is fairly slow (32, 50). Thus, the differentiation between the two processes was defined by the filtration slope between 3 and 8 min (Fig. 1). In our earlier studies on venous compliance we used a linear compliance model since the studied transmural pressure interval was quite high (18 to 51 mmHg) (43). In the present investigation, a lower pressure interval was used (9-36 mmHg), where the volume-pressure relationship was clearly non-linear (P < 0.05, Fig. 2A). Accordingly, a non-linear regression equation model characterising the volume-pressure curve was used (Fig. 2B, Methods section). In contrast to others, we did not increase the intravascular pressure by means of a thigh cuff (6, 17, 20, 34, 39, 47), but used application of negative pressure around the leg caused by LBNP.

Lower body negative pressure (LBNP) leads to a decrease in venous return, central blood volume and subsequently to a drop in arterial pressure (4, 5, 10, 58), and the venous compartment in the legs, rather than the pelvic or abdominal region, seems to have a hemodynamic impact during LBNP (16). This in turn elicits an increased sympathetic response with higher peripheral resistance and increased heart rate (4, 5, 10, 44). In studies on men, a greater calf venous compliance has been linked to an increased venous capacitance response, which seems to have an impact on cardiovascular responses to orthostatic stress and orthostatic tolerance (43, 56). Women are more susceptible to orthostatic stress than men (4, 10, 13, 40, 51, 58), and in accordance with some findings in the arterial tree, it may be
hypothesised that women have greater venous compliance in the lower limbs predisposing to orthostatic intolerance (52).

We found a reduced calf venous compliance \( (C_{cap}) \) in women however, assessed as the slope of the \( C_{cap} \)-pressure curve as well as \( \beta_1 \) and \( \beta_2 \), in analogy with other recent studies (Fig. 2B, Table 2) (34, 39). The \( C_{cap} \) in the legs is linked to the capacitance response, and the lower \( C_{cap} \) in women explains their lower capacitance response in the present study (Fig. 2) (43, 56). The possible importance of the venous capacitance of the legs in orthostatism has earlier been addressed (16). There might be differences between sexes confounding such a link however (4). First, pelvic blood pooling during LBNP seems to be higher in women which could contribute to the differences in cardiovascular reactions to LBNP (59), although the abdominal-pelvic region seems to be of much less importance than the legs (16). Second, the body composition varies between sexes with a higher relative soft tissue as well as skeletal muscle volume in the lower than in the upper part of the body in women (24, 41), which means that a larger proportion of the circulating blood volume may be pooled in the lower part of the body. Third, blood volume is lower in women compared to men although this difference may be due to differences in body size (38). Other factors of importance for the susceptibility to orthostatic stress in women might be reduced cardio-vagal baroreflex gain, peripheral resistance, muscle sympathetic nerve activity (MSNA), and/or different adrenergic receptor sites, response time and duration to orthostatic stress (3, 7, 30, 51). Larger decrease in stroke volume has also been found in women as compared to men (4, 9).

During LBNP the central hypovolemia elicits baroreceptor deactivation with a concomitant sympathetic response and increase in circulating norepinephrine levels (4, 5, 9, 44). Although in vivo experiments on human hand veins have provided evidence for sympathetic constrictor
responses both via α1-adrenoreceptor agonists and neuropeptide Y (29), no evidence exists
to our knowledge that active constriction of capacitance vessels in skeletal muscle (40-50% of
the body weight) provides an important target of sympathetic responses. Thus the main part of
the venous reservoir is adjusted simply by means of passive changes. Arteriolar resistance
however increases by sympathetic stimulation of the arterial smooth muscle and the flow
tends to decrease (4, 9, 13, 44, 51), leading to a decreased pressure gradient from capillaries to
large veins as well as a decrease in small vein pressure (48). This train of events probably
occurs during LBNP, even if no change in large venous pressure is detected (1). Small
changes in intravenous pressure owing to changes in blood flow will have an effect on venous
volume, and unstressed venous volume may thus decrease as shown in the extremities using
ischemic handgrip or LBNP (17, 39). Basal arterial inflow to the lower limb seems to be
similar in women and men (21, 28), and a majority of studies have found no sex difference in
arterial vasoconstriction (9, 10, 13, 58) although men may respond with greater
vasoconstriction during LBNP (7). A greater vasoconstriction in men, however, would have
led to an underestimation of the differences in capacitance response in the present study.
Further, care was taken to place the midpoint of the calf 5 cm below heart level in all subjects,
minimizing potential differences in unstressed volume and in accordance no difference in \( \beta_0 \)
between women and men was found (Methods section, Table 2). The underlying sex
differences in the venous walls are at present unknown, as well as the distribution of
compliances within the smallest veins as opposed to the largest ones. Structural differences
may be linked to sex related hormonal influence on collagen-elastin ratio as well as wall
thickness. Estrogen receptors are known to exist in smooth muscle cells, and estrogens have
been shown to affect cellular transcription of elastin and collagen (25, 37).
It is of interest to note that the sex difference in the C$_{cap}$-pressure curves was more marked at low transmural pressures, where even small transmural pressure changes in the peripheral veins are followed by substantial differences in volume (Fig. 2B). This part of the curve is the principal culprit for rapid mobilization of venous blood to the effective circulating blood volume during hypovolemic circulatory stress, indicating a less effective compensatory response in women (44). This is in accordance with the findings of Meendering et al (2005) and Monahan and Ray (2004) who found a higher calf venous compliance in men at an assumed zero mmHg transmural pressure. With increasing transmural pressure, C$_{cap}$ decreases in both women and men (Fig. 2B) (34, 39). The sex difference in C$_{cap}$ diminished with increasing transmural pressure and at higher transmural pressures no difference was found (Fig. 2B). At transmural pressures relevant to quiet standing or head up tilt (HUT), women may in fact have a higher C$_{cap}$ than men (Results section, Fig. 2B), in contrast to the findings by Meendering et al (2005) and Monahan and Ray (2004), where men consistently seemed to have a higher venous compliance up to 60 mmHg. The reasons for this difference between the studies are unknown, but might be due to methodological variations. The cuff method used by Meendering et al (2005) and Monahan and Ray (2004) equals cuff pressure with venous pressure, assuming a 100% transmission of applied pressure to the underlying tissue. This is by no means certain, and transmission especially to the deep underlying tissue in the thigh may be overestimated (18). Further, thigh volume might differ between sexes, which could affect the found differences in calf venous compliance at transmural pressures relevant to HUT or quiet standing. Transmission of externally applied negative pressure on the other hand, seems equally transmitted to all vessel segments irrespective of muscle depth in the studied calf tissue (43).
Huxley et al (2005) used a large experimental animal model and found increased micro-vessel permeability only in females after administration of adenosine (23). To the best of our knowledge, no previous study has assessed sex differences in capillary fluid filtration and CFC in humans. In line with our hypothesis, the capillary fluid filtration to the tissue of the calf as well as CFC was increased in women compared to men (Figs 3 and 4). The CFC measured in male subjects was of similar magnitude as described earlier with similar techniques (26, 36). This low CFC of 0.003-0.004 (ml 100ml⁻¹ min⁻¹ mmHg⁻¹) may have decreased from its basal level due to local myogenic as well as axon reflex responses to the increased transmural pressure (19, 31). Further, CFC may have been affected not only by local increase in transmural pressure but also by increased sympathetic discharge in response to the reduced central blood volume during LBNP. Thus, CFC might have deteriorated to some extent from the value determined by the local transmural pressure changes only, due to an increase in pre/post capillary resistance ratio, and a concomitant decrease in capillary pressure as well as opening of pre-capillary sphincters due to sympathetic activation (27, 36). However, differences in sympathetic activation (e.g. high as opposed to low LBNP, or differences in peripheral resistance) do not seem to affect CFC in the leg during LBNP (Fig. 4) (26), and when CFC is studied with local techniques only, similar values as in the present study have been found, indicating only minor importance of the sympathetic discharge (2, 11, 33). The increased capillary filtration in women may be due to higher levels of estrogen and its effect on the microcirculation (45, 54, 55). Tollan et al. (1992) proposed a direct effect of estrogen on capillary protein permeability, which increases filtration capacity (55).

Furthermore, the vasodilatory effect of estrogen may increase capillary pressure and facilitate leg capillary filtration during LBNP (12, 22). Atrial natriuretic peptide (ANP) affects capillary filtration by increasing CFC and/or protein permeability (14, 60), and estrogen augments the ANP effect on CFC (54).
In order to elucidate the effect of capillary filtration on venous compliance calculations, Halliwill et al (1999) compared a short period of thigh cuff stasis (4 min) with a longer period (8 min) with presumably larger amount of interstitial fluid accumulation due to capillary fluid filtration in the calf. The volume-pressure relationship was characterized without separating capillary filtration from the capacitance response during one minute of linear cuff deflation from 60 to 10 mmHg and no impact of filtration on venous compliance was found (17). It might be argued that changing intravenous pressure at a rate of 1 mmHg/s over a single minute would not allow much of the filtered tissue fluid to re-enter the circulation. A relatively small group (5 women, 4 men) was studied however making a putative effect difficult to detect. It has been suggested that capillary filtration coefficient, CFC, is high in humans especially at low transmural pressure gradients (31). Our data show that capillary filtration (Fig. 3), as well as V_{filtr} was larger in women during LBNP, and V_{filtr} increased its contribution to total calf volume change with increasing LBNP levels in both sexes (Results section, Fig. 5). In fact, capillary filtration augmented the volume increase further by roughly 50 percent during a similar time of increased pressure as used by Halliwill et al (1999). When V_{filtr} was not accounted for and not excluded from the total calf volume increase, the calculated compliance (C_{tot}) differed from C_{cap} especially in women (β_1, 27%, P < 0.05), β_2, -43%, P < 0.05), but to a lesser extent also in men (β_1, 15%, P < 0.05, β_2, -21%, P = 0.07), and the sex difference found in C_{cap} disappeared (Results section, Table 2 and 3, Fig. 6). The greater decrease of β_2 in women indicate a more pronounced effect of capillary filtration on C_{cap} in women, who tend to increase the total calf volume more rapidly at higher transmural pressure levels than men, probably caused by sex differences in CFC (Figs 3, 4, 5 and 6). The large calf volume increase caused by capillary filtration shows high potential for fluid re-absorption even during such a short period of time used by Halliwill et al (1999), Meendering
(2005) and Monahan and Ray (2004). \( V_{\text{filtr}} \) contributes to the central hemodynamic load independently from the pooling of blood in the capacitance vessels, and not only \( V_{\text{cap}} \) but also \( V_{\text{filtr}} \) must be addressed concerning the orthostatic stimuli, with \( V_{\text{filtr}} \) adding substantial volume during LBNP exposure, especially in women (Results section, Figs 1 and 5). Despite the fact that \( V_{\text{cap}} \) was higher in men, no sex difference in calf volume increase was found because of the higher \( V_{\text{filtr}} \) in women (Figs 2 and 5).

In conclusion, calf venous compliance was significantly reduced in women compared to men with a concomitant reduction in capacitance response during LBNP, indicating a lower central hypovolemic stimulus in women. However, these differences were only seen at lower transmural pressures, while at higher transmural pressures no sex differences were seen. Further, interstitial fluid accumulation due to capillary fluid filtration was larger in women than in men, probably due to higher CFC. Calf volume increase (capacitance response + total capillary filtration) during LBNP was similar in women and men, which points towards comparable central hypovolemic stimulus in women and men. Finally the sex-related differences in venous capacitance (\( V_{\text{cap}} \)) and venous compliance (\( C_{\text{cap}} \)) were hidden if the contribution of capillary filtration was not accounted for.
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References


Figure legends

Fig. 1. Original tracing illustrating tissue volume changes in the calf in response to LBNP 44 mm Hg in a 23-year-old woman. Initial rapid increase in volume reflects capacitance response of 2.34 ml 100ml⁻¹. The subsequently much slower but continuous increase reflects net capillary filtration of plasma fluid into tissue of 0.163 ml 100ml⁻¹ min⁻¹.

Fig. 2. (A) Venous capacitance calf (V<sub>cap</sub>) in relation to transmural pressure and the curve made by the regression equation in women (dashed lines), and men (solid lines), mean ± SE. The capacitance response was higher in men than in women (P < 0.05). (B) The corresponding compliance (C<sub>cap</sub>)-pressure curves, where β1 and the slope of the C<sub>cap</sub>-pressure curve were larger in men (P < 0.05). It is obvious that C<sub>cap</sub> is dependent on transmural pressure, and C<sub>cap</sub> is significantly higher in men than in women at low transmural pressures (P < 0.05). The C<sub>cap</sub> curves cross at approximately 28 mmHg, and at higher transmural pressures, women seem to have greater C<sub>cap</sub>, without reaching significance up to 36 mmHg.

Fig. 3. Capillary fluid filtration of calf in response to LBNP in women and men. Capillary fluid filtration increased with increasing LBNP, being larger in women (white bars) than men (black bars) at LBNP 11 and 22 mmHg as well as overall (P < 0.05).

Fig. 4. Capillary fluid coefficient (CFC) of calf in relation to transmural pressure changes evoked by LBNP in women and men. CFC was similar irrespective of LBNP level. CFC was however larger in women (white bars) than men (black bars) at LBNP 11 and 22 mmHg as well as overall (P < 0.05).
Fig. 5. Total volume increase ($V_{\text{tot}}$) of calf in response to LBNP in women (white bars), and men (black bars). The contribution of venous capacitance (lower part of bars, $V_{\text{cap}}$) and total filtration (upper part of bars, $V_{\text{filtr}}$) to $V_{\text{tot}}$ are also shown. No sex difference in $V_{\text{tot}}$ was seen. $V_{\text{filtr}}$ was larger in women however, and contributing more to $V_{\text{tot}}$ at all LBNP levels ($P < 0.001$).

Fig. 6. Volume increase of calf in relation to transmural pressure changes evoked by LBNP, taken either from the capacitance response ($V_{\text{cap}}$, solid lines), or from the total volume increase ($V_{\text{tot}} = V_{\text{cap}} + \text{total capillary filtration}, V_{\text{filtr}}$, dashed lines) in (A) women (circles) and (B) men (squares). The corresponding venous compliance ($C_{\text{cap}}$)- and ($C_{\text{tot}}$)-pressure curves in women and men are shown below. While both $\beta_1$, $\beta_2$ and slope changed significantly in women when comparing $C_{\text{cap}}$ with $C_{\text{tot}}$ ($P < 0.05$), only $\beta_1$ changed in men ($P < 0.05$) (see Table 3 for details).
Figure 1
Figure 2

A

Capacitance response, calf (ml 100ml⁻¹ mmHg⁻¹)

B

$C_{\text{cap}}$, calf (ml 100ml⁻¹ mmHg⁻¹)

Transmural pressure (mmHg)
Figure 3
Figure 4
Figure 5
Figure 6

A. Women

B. Men

Volume increase, calf (ml 100ml⁻¹)

C and C_total, calf (ml 100ml⁻¹ mmHg⁻¹)

Transmural pressure (mmHg)
Table 1

Table 1. Demographic resting values in women and men.

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th>Men</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>22.9±0.5</td>
<td>23.2±0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171±2</td>
<td>181±1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>63±2</td>
<td>72±1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BMI (kg m⁻²)</td>
<td>21.6±0.5</td>
<td>21.8±0.4</td>
<td>NS</td>
</tr>
<tr>
<td>HR (beats min⁻¹)</td>
<td>61±2</td>
<td>56±2</td>
<td>NS</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>107±1</td>
<td>116±2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>61±2</td>
<td>60±1</td>
<td>NS</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>76±1</td>
<td>78±1</td>
<td>NS</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>46±1</td>
<td>56±2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure.
Table 2

Table 2. Regression equation and the parameters $\beta_0$, $\beta_1$, and $\beta_2$.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Regression Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>$\Delta$ limb volume = 0.014±0.14 + (0.086±0.018* x (transmural pressure)) + (-0.00067 ±0.00036* x (transmural pressure)^2)</td>
</tr>
<tr>
<td>Men</td>
<td>$\Delta$ limb volume = -0.142±0.054 + (0.130±0.0098 x (transmural pressure)) + (-0.00145 ±0.00017 x (transmural pressure)^2)</td>
</tr>
</tbody>
</table>

Values are means ± SE. $\Delta$ limb volume = $\beta_0 + \beta_1 x$ (transmural pressure) + $\beta_2 x$ (transmural pressure)^2. * $P < 0.05$ women vs. men.
Table 3

<table>
<thead>
<tr>
<th></th>
<th>( \beta_1 )</th>
<th>( \beta_2 )</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>C(_{\text{cap}})</td>
<td>0.086±0.018* * #</td>
<td>-0.00067±0.00036* #</td>
</tr>
<tr>
<td></td>
<td>C(_{\text{tot}})</td>
<td>0.109±0.022</td>
<td>-0.00038±0.00046</td>
</tr>
<tr>
<td>Men</td>
<td>C(_{\text{cap}})</td>
<td>0.130±0.0098* *</td>
<td>-0.00145±0.00017</td>
</tr>
<tr>
<td></td>
<td>C(_{\text{tot}})</td>
<td>0.149±0.012</td>
<td>-0.00115±0.00020</td>
</tr>
</tbody>
</table>

Values are means ± SE. * P < 0.05 between C\(_{\text{cap}}\) and C\(_{\text{tot}}\) within gender, * # P < 0.05 in C\(_{\text{cap}}\) and C\(_{\text{tot}}\) between genders. C\(_{\text{cap}}\), compliance derived from venous capacitance, V\(_{\text{cap}}\). C\(_{\text{tot}}\), compliance derived from V\(_{\text{tot}}\) (venous capacitance + total filtration).