Original communication

Intermittent negative pressure influences popliteal artery shear rate during supine and sitting postures

Nigel A. Callender[1,2,3], Lars Øivind Høiseth[1,4], Iacob Mathiesen[3], and Jonny Hisdal[1,2]

[1] Faculty of Medicine, University of Oslo, Norway
[2] Department of Vascular Surgery, Oslo University Hospital, Norway
[3] Otivio AS, Oslo, Norway
[4] Department of Anaesthesia and Intensive Care Medicine, Oslo University Hospital, Norway

Summary: *Background:* Intermittent negative pressure is an emerging treatment for lower limb vascular disease but the specific physiological effects, particularly upon large artery haemodynamics are unclear. This study examined the influence of intermittent negative pressure upon popliteal artery shear rate during both supine and sitting postures. *Participants and methods:* Eleven healthy participants (5 female; age: 28.3 ± 5.8 y; weight: 69.6 ± 9.8 kg, height: 1.75 ± 0.07 m) received intermittent negative pressure (–37 mmHg; 9.5-sec on, 7.5-sec off), upon the lower leg during both supine and sitting postures. Popliteal artery blood flow and shear rate were recorded (duplex ultrasound), accompanied by heart rate (3-lead ECG) and blood pressure (volume clamp method). *Results:* Compared to sitting, a supine posture led to greater mean shear rate during baseline (supine: 21[9]; sitting: 17[13] sec^{-1}; all median [IQR]) and negative pressure phases (supine: 24[15]; sitting: 17[14] sec^{-1}; both $p<0.05$). While supine, negative pressure raised mean shear rate above baseline levels ($p<0.05$) and reduced it upon return to atmospheric pressure ($p < 0.05$). In sitting, mean shear rate only differed from baseline at the points of peak and minimum shear (peak:18[17]; minimum: 10[9] sec^{-1}; both $p<0.05$). Shear pattern (oscillatory shear index) showed changes from baseline during both postures ($p<0.05$), but was not different between postures. Conclusions: Intermittent negative pressure influenced lower limb popliteal artery shear rate during both the supine and sitting postures, the effect was greater while supine. Fluctuation in shear pattern seen during both positions may account for positive clinical effects observed following intermittent negative pressure treatment. These findings are framed against previous work investigating clinical populations. Future work should investigate any differences in lower limb haemodynamics and markers of endothelial function among patients with vascular disease.

Keywords: Negative pressure, atherosclerosis, non-invasive treatment, intermittent negative pressure, arterial shear

Introduction

Peripheral arterial disease remains a leading cause of morbidity and mortality and its burden is growing globally [1]. Non-invasive treatment options particularly among those with severe disease remain limited, but mechanical therapies do offer a potential option for some patient groups. Among these, intermittent pneumatic compression is established, but recently, intermittent negative pressure (INP) has emerged as a novel therapeutic option [2, 3]. While intermittent pneumatic compression is known to elicit increases in blood velocity in the popliteal artery [4], the overall clinical effect may result from modulation of intravascular shear forces [5]. Similar mechanisms might underpin the action of INP, but at present little work has described the acute physiological changes associated with its use. This is despite possible benefits in the context of non-operative higher-grade or critical limb ischaemia [3] owing to the non-compressive nature of the treatment upon the skin and lower pressure differential involved.

Endothelial dysfunction is associated with the development of atherosclerosis and is mediated in part by vascular shear forces [6]. Shear, the frictional force at the interface between blood and endothelium can be estimated by calculation of shear rate (blood velocity/diameter, without accounting for haematocrit; [7]). This varies according to anatomic site and posture [8], age [9], physiological load [10], and to passive stimuli such as isolated limb heating [11]. Classically, increased anterograde shear rate is thought to improve endothelial function, while greater retrograde shear, or shear oscillation has been thought to hold a deleterious effect [6, 11, 12]. Recently however,

fluctuation in shear pattern has emerged as a potentially important mediator of vascular function [13, 14, 15], even in the context of an unchanged mean shear rate during the therapy cycle as a whole [15].

Negative pressure enclosing a limb segment transmits to both superficial and deep compartments [16] and transiently influences pressure within the venous system [17]. It is through changes in the arteriovenous gradient and local increases in vascular resistance [18] that oscillation in conduit artery blood flow and shear rate may occur with INP [15]. Intermittent negative pressure is already known to induce fluctuations in brachial artery shear rate [15] in addition to eliciting cyclic changes in the superficial microvasculature of the leg [19, 20], however, the pattern in the more atherosclerosis-prone lower limb conducting vessels remains to be investigated. Indeed, given the greater ability of the leg vessels to resist pressure-induced changes in diameter and flow [21, 22], regional differences in INP response might be anticipated.

Despite the growing body of work demonstrating positive clinical effects of INP [19, 20], there remains a need to specifically define the underlying mechanisms in the lower limb. Furthermore, postural change has been shown to alter lower limb shear responses [8], and given the use of INP devices largely during sitting, knowledge of any interaction between INP and posture is warranted. Therefore, the aims of this study were first, to investigate the acute influence of INP upon popliteal haemodynamics and shear rate, and second, to ascertain whether postural change alters such responses given the greater constraint upon blood flow typically found during more upright positions.

Participants and methods

Participants

Eleven participants were included in the study (6 males, 5 females; age: 28.3 ± 5.8 y; weight: 69.6 ± 9.8 kg; height: 1.75 ± 0.07 m). All were free from conditions or medications influencing the cardiovascular system and did not use nicotine-containing products. Participants also refrained from caffeine (>8 h), alcohol and heavy exercise (>24 h) prior to testing. Written, informed consent was provided following approval from the local regional ethics committee (REK: 2021/323184 and 2022/466181), and all procedures aligned with the *Declaration of Helsinki* except for registration within a database.

Procedure

Following medical screening and basic anthropometric measures, one leg was placed within a commercially available INP chamber coupled to its pump unit. After 20-min of quiet rest, blood flow in the popliteal artery, systemic blood pressure (BP), heart rate (HR), chamber pressure were recorded for a baseline period of 60–100 sec, followed by five cycles of INP (9.5-sec on-phase [–37 mmHg], 7.5-sec off-phase [ambient atmospheric pressure]). These measures were undertaken in both the supine and sitting positions, with a minimum 10-min stabilisation period observed between any postural change. Laboratory temperature was 24.5 ± 1.0° C and ambient atmospheric pressure 753 ± 8 mmHg.

Measures

Anthropometry and systemic cardiovascular measures

Height (m) and weight (kg; Diagnostic XXL; Medel International, Milan, Italy) were measured using standard methods. Heart rate was recoded continuously via 3-lead ECG (Dual Bio Amp; ADInstruments, Dunedin, New Zealand) in addition to beat-by-beat blood pressure (Nexfin; BMEYE, Amsterdam, Netherlands) during each baseline and intervention period.

Negative pressure

Intermittent negative pressure was delivered using a commercially available clinical treatment system comprising a lower leg chamber and pressure control unit (FlowOx 2.0; Otivio, Oslo, Norway). Pressure within the chamber was reduced relative to ambient atmospheric pressure (–37 mmHg gauge pressure) in fixed cycles of 9.5 sec on, 7.5 sec off with one complete negative pressure period and its successive ambient pressure period representing a complete INP cycle. Ramp time at onset to 95% plateau pressure was 1.6 sec, with an offset time of 1.0 sec. A well-fitting, low compression, thermoplastic elastomer seal interfaced the leg and chamber ~10 cm below the popliteal crease, allowing easy access and steady probe positioning during both postures. Chamber pressure (Stranden Manometer; Stranden Instruments, Ålesund, Norway) was recorded continuously during all test sequences.

Blood flow

Using duplex ultrasound (Vivid E95; GE Healthcare, Horten, Norway), blood flow and arterial diameter (longitudinal plane) were recorded continuously using a 9 MHz linear probe (9L; GE Healthcare) at the popliteal artery. Sample volume was set to the full width of the vessel and an insonation angle of <60 degrees maintained throughout. On-screen anatomical landmarks were identified to ensure a similar recording site between both postures. Machine settings were maintained as closely as possible between supine and sitting positions and both doppler and 2D signals were optimised throughout.

Ultrasound data were analysed offline, beat-by-beat, using semi-automated tracing of the Doppler signal (EchoPac; GE Healthcare). Diameter was determined from the longitudinal B-mode image using the caliper function and the average of three measures taken during end-diastole between the intimal edge of the leading wall to that of the far wall. No

difference in vessel diameter was identified between negative and atmospheric pressure phases, therefore a mean of all cardiac cycles combined across all pressure cycles was included as vessel diameter during analyses. Ultrasound images were captured live within Labchart, using the software's video-grab function, allowing alignment of the ultrasound recordings with the remaining measures.

Signal acquisition and analyses

All signals were captured using an analogue to digital convertor (PowerLab 16/35; ADInstruments, Dunedin, New Zealand), sampling at 1 KHz and recorded within the proprietary software (Labchart Pro; ADInstruments). Ultrasound images were captured live within Labchart, using the software's video-grab function, allowing temporal alignment of the ultrasound recordings with the remaining measures. Data from Labchart and ultrasound doppler measures were exported on a beat-to-beat basis, gated to the R-peak of the ECG, and combined in Microsoft Excel. Baseline data were taken as the average of each 60–100 sec resting period preceding each intervention phase. Thereafter, three to five cycles of negative pressure and their successive atmospheric pressure phases were extracted and averaged for each participant. As an indication of peak and minimum shear, during each negative pressure phase, the three sequential cardiac cycles corresponding to peak blood flow/mean shear rate (Peak) were identified and additionally, the three sequential cycles during the subsequent atmospheric pressure phase representing minimum flow/mean shear rate (Minimum) were identified and averaged for each participant and included for analysis.

Blood flow was calculated using the formula: $\dot{Q} = A \cdot (\text{TAMax}/2) \cdot 60$ where $\dot{Q}$ = blood flow, A = vessel cross sectional area (cm^2) and TAMax = the time averaged maximum envelope of the velocity trace ($cm \cdot s^{-1}$). Shear rates (s^{-1}) were calculated using the formula (4·blood velocity)/diameter. For each cardiac cycle, mean, peak anterograde, and peak retrograde shear rate was calculated from mean (TAMax/2), maximum (peak systolic velocity), and minimum (minimum diastolic velocity) blood velocities respectively ($cm \cdot sec^{-1}$), and end-diastolic arterial diameter (cm). Oscillatory shear index (OSI) provides an index of shear pattern, where 0.5 indicates an equal anterograde-retrograde contribution, with lower values indicating a more anterograde-dominant pattern of shear. This was calculated as OSI = peak retrograde shear / (peak anterograde + peak retrograde shear) [23].

Quantile-quantile plots and histograms were used to assess normality. In view of the small sample size and deviation of some variables from a normal distribution, between-phase, comparisons were undertaken using Friedman tests, with Holm-Bonferroni corrected Wilcoxon tests used for *post-hoc* analyses. Between-posture comparisons were made using Wilcoxon tests. Non-normally distributed data are presented as median [IQR], with normally distributed data presented as mean ± SD and level of statistical significance was set at $p<0.05$.

Results

Eleven participants were included in the final analysis. Negative pressure did not elicit any change in central cardiovascular variables during any phase, within either posture (Figure 1, Electronic supplementary material 1; all $p>0.05$). Heart rate, mean and diastolic arterial pressure were significantly different between postures ($p<0.05$). Differences in systolic pressure were only statistically significantly at the baseline timepoint (Figure 1; ESM 1).

Popliteal artery blood flow was statistically significantly different between timepoints in the supine (Baseline: 43 [17], Negative pressure phase: 60 [19], Atmospheric pressure phase: 33 [11] $ml \cdot min^{-1}$; Friedman test = 38.764, $p<0.001$) and sitting postures (Baseline: 33 [29], Negative pressure phase: 34 [28], Atmospheric pressure phase: 28 [21] $ml \cdot min^{-1}$; Friedman test = 35.564, $p<0.05$; *Post hoc* test results, Table I), but was only attained statistical significance between postures during the negative pressure phase and peak shear timepoint (both $p<0.05$; Table I).

Statistically significant differences to baseline measures were present during both the supine and sitting postures for mean shear rate (Supine Baseline: 21 [9], Negative pressure: 24 [15], Atmospheric pressure: 15 [8], Peak: 25 [19], Minimum: 14 [5]; Friedman test = 37.309, $p<0.05$; Sitting Baseline: 17 [13], Negative pressure: 17 [14], Atmospheric pressure: 14 [11], Peak: 18 [17], Minimum: 10 [9]: Friedman test = 32.291, $p<0.05$), anterograde shear rate (Supine Baseline: 454 [203], Negative pressure: 429 [155], Atmospheric pressure: 437 [171], Peak: 432 [157], Minimum: 430 [170]: Friedman test = 25.818, $p<0.05$; Sitting Baseline: 212 [77], Negative pressure: 207 [85], Atmospheric pressure: 199 [88], Peak: 205 [93], Minimum: 430 [170]; Friedman test = 12.582, $p<0.05$), retrograde shear rate (Supine Baseline: 211 [99], Negative pressure: 147 [59], Atmospheric pressure: 178 [102], Peak: 135 [72], Minimum: 160 [113]; Friedman test = 26.327, $p<0.05$; Sitting Baseline: 85 [32], Negative pressure: 81 [29], Atmospheric pressure: 88 [29], Peak: 74 [30], Minimum: 101 [32]; Friedman test= 27.418, $p<0.05$; *post-hoc* results see Figure 2) and oscillatory shear index (Supine: Friedman test = 33.382, $p<0.05$; Sitting: Friedman test = 35.418, $p<0.05$) *Post-hoc* results, Table I). Anterograde and retrograde shear rate (Figure 2) were significantly different between postures at all timepoints ($p<0.05$). Differences in mean shear rate between postures attained statistical significance ($p<0.05$) at all timepoints except during the atmospheric pressure phase and the minimum shear timepoint ($p=0.240$ and 0.206 respectively). Oscillatory shear index did not vary between postures at any timepoint (all $p>0.05$; Table I). Popliteal artery diameter was greater in the sitting, relative to the supine position (supine: 5.51 ± 0.74, sitting: 5.94 ± 0.73 mm; $p=0.010$; mean ± SD, data normally distributed, comparisons using related-samples *t*-tests), and was not influenced by INP (supine: negative pressure 5.53 ± 0.73 *vs.* atmospheric pressure 5.49 ± 0.76 mm, $p=0.067$; sitting: negative pressure 5.94 ± 0.74 *vs.* atmospheric pressure 5.93 ± 0.47 mm, $p=0.810$; all mean ± SD, data normally

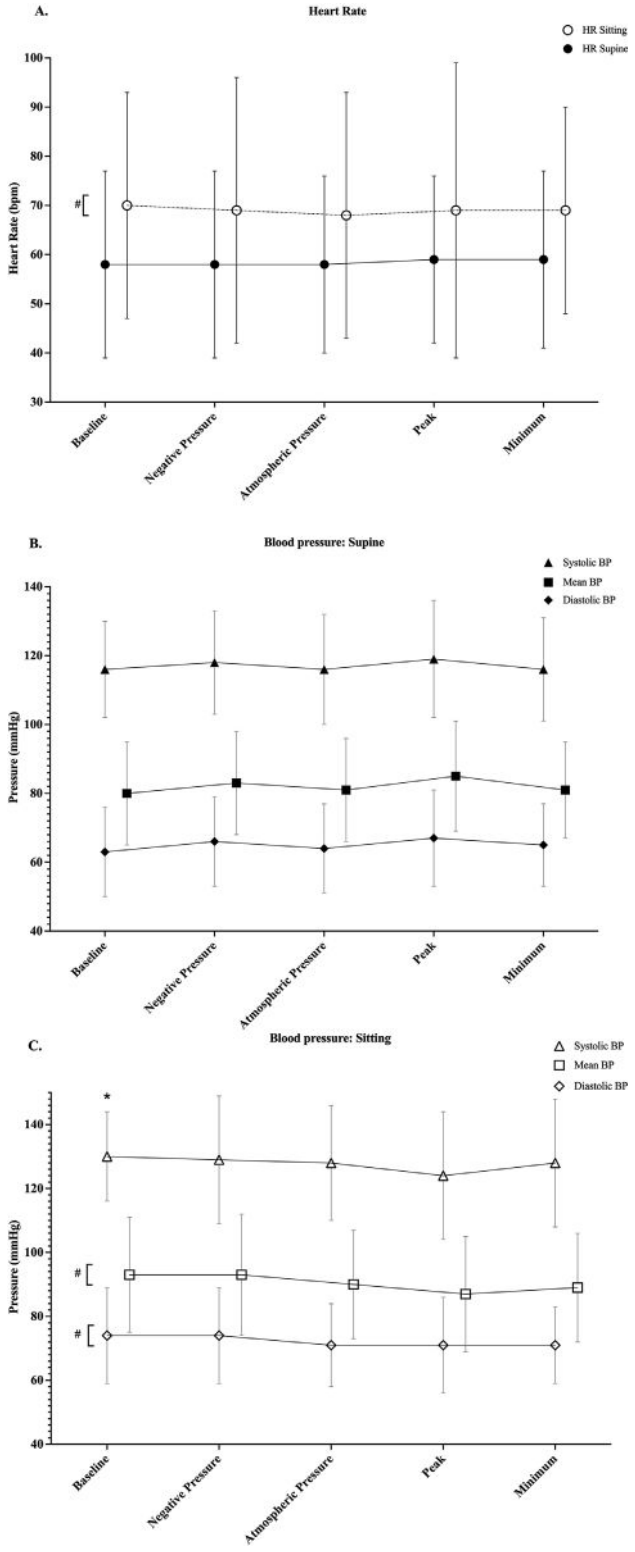

Figure 1. Central cardiovascular variables during intermittent negative pressure in the supine and sitting postures. Central cardiovascular variables were higher in the sitting than supine posture but did not vary between key phases and timepoints. Solid symbols: Supine posture, open symbols: Sitting posture. Panel A: Heart rate (BPM; beats per minute). Panel B: Supine blood pressure (BP). Panel C: Sitting blood pressure (BP; mmHg). All values median [interquartile range]. Full data is presented in ESM 1. #Significantly different to supine (all p<0.05), *denotes only baseline values significantly different to supine (p<0.05).

Table I. Blood flow and oscillatory shear index at key points during the intermittent negative pressure cycle during supine and sitting postures.

	Popliteal Flow (ml · min^{-1}) n = 11	OSI (AU) n =11
	Supine	
Baseline	43 [17]	0.28 [0.05]
Range	*29–65*	*0.12–0.34*
Negative Pressure	60 [19]*	0.25 [0.04]*
Range	*31–72*	*0.13–0.34*
Atmospheric Pressure	33 [11]$^{*\$\P}$	0.30 [0.07]$^{*\$\P}$
Range	*19–51*	*0.21–0.35*
Peak	68 [23]$^{*\$}$	0.25 [0.04]*
Range	*30–88*	*0.14–0.33*
Minimum	30 [8]$^{*\$\P}$	0.30 [0.09]$^{*\$\P}$
Range	*19–46*	*0.24–0.36*
	Sitting	
Baseline	33 [29]	0.26 [0.08]
Range	*18–80*	*0.18–0.37*
Sig. level vs. Supine	p = 0.206	p = 0.398
Negative Pressure	34 [28]	0.27 [0.08]¶
Range	*20–72*	*0.18–0.38*
Sig. level vs. Supine	p = 0.019	p = 0.365
Atmospheric Pressure	28 [21]$^{\$\P}$	0.28 [0.09]$^{*\$\P}$
Range	*18–74*	*0.19–0.38*
Sig. level vs. Supine	p = 0.898	p = 0.123
Peak	37 [39]$^{\$}$	0.25 [0.07]$^{*\$}$
Range	*21–78*	*0.15–0.38*
Sig. level vs. Supine	p = 0.019	p = 0.520
Minimum	26 [15]$^{*\$\P}$	0.29 [0.10]$^{*\$\P}$
Range	*17–63*	*0.20–0.39*
Sig. level vs. Supine	p = 0.765	p = 0.083

Notes. Popliteal artery blood flow (millilitres per minute) and oscillatory shear index (OSI: oscillatory shear index; arbitrary units) at key points during the intermittent negative pressure cycle. Baseline: Resting baseline period. Negative pressure: Mean for the full negative pressure phase. Atmospheric pressure: Mean for the full atmospheric pressure phase. Peak: Point of greatest mean shear rate during the negative pressure phase. Minimum: Point of lowest mean shear rate following the return to negative pressure. All median [interquartile range]. Range: Minimum to maximum value. *Significantly different to Baseline $p < 0.05$. $^{\$}$Significantly different to Negative Pressure $p < 0.05$. ¶Significantly different to Peak $p < 0.05$.

distributed, comparisons using related-samples *t*-tests, Figure 3).

Discussion

Among a group of healthy participants, we investigated the influence of intermittent negative pressure upon popliteal artery blood flow and shear rate. Additionally, this was examined between the supine and sitting postures, where

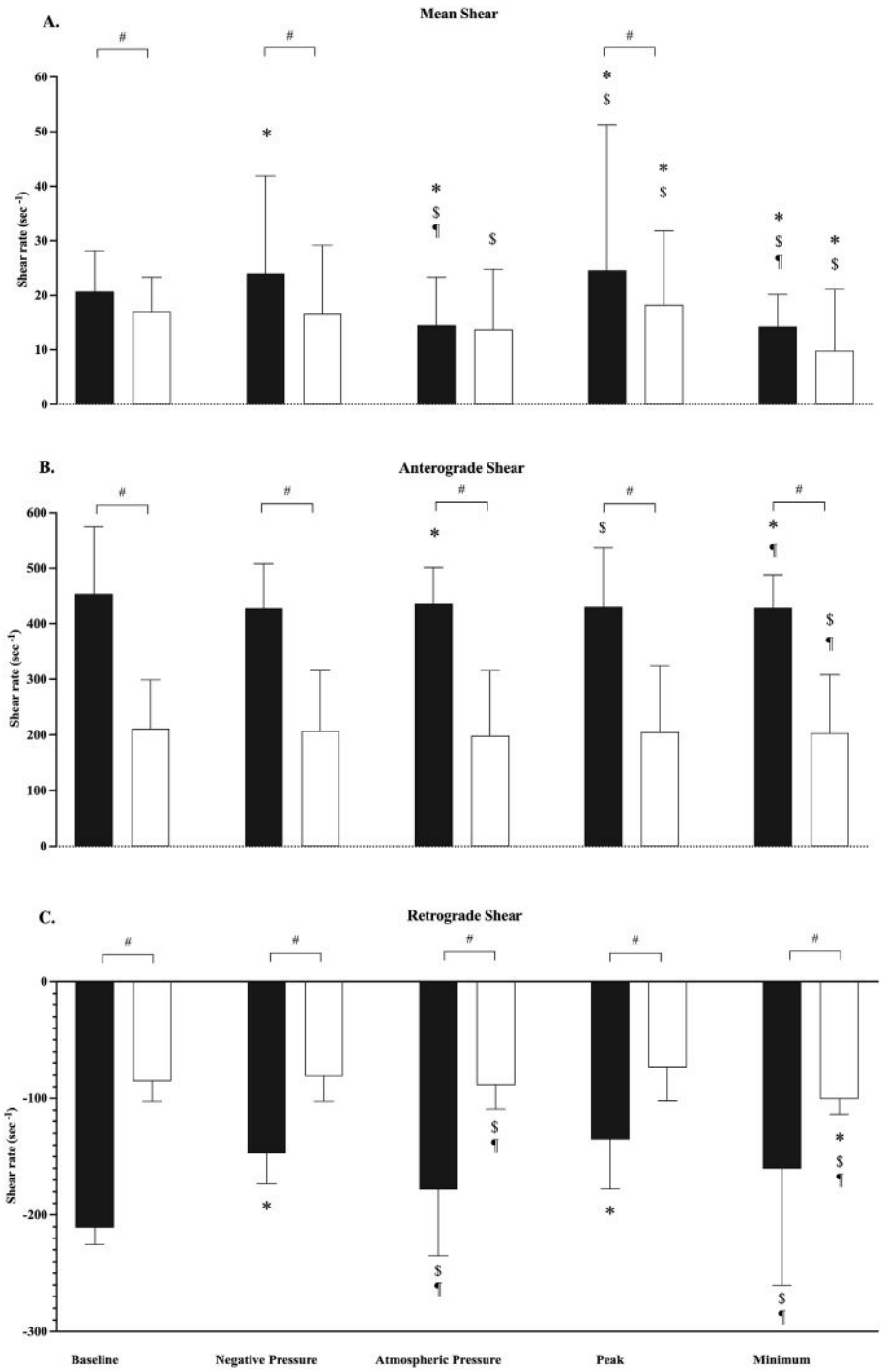

Figure 2. Shear rate between postures and phases of the intermittent negative pressure cycle. Panel A: Mean shear rate, Panel B: Anterograde shear rate and Panel C: Retrograde shear rate (all sec^{-1}). Baseline: Resting baseline period. Negative pressure: Median for the full negative pressure phase. Atmospheric pressure: Median for the full atmospheric pressure phase. Peak: Point of greatest mean shear rate during the negative pressure phase. Minimum: Point of lowest mean shear rate following the return to negative pressure. Solid bars: Supine posture, open bars: Sitting posture. *Significantly different to baseline ($p<0.05$), $ significantly different to negative pressure ($p<0.05$), ¶ significantly different to peak ($p<0.05$), # significantly different between postures ($p<0.05$).

changes in peripheral vascular resistance produced differing constraints upon blood flow. During the baseline and negative pressure phases, mean, anterograde and retrograde shear rate were greater in the supine than sitting position. During the negative pressure phase, mean shear was only elevated above baseline levels in the supine posture. Importantly and regardless of body position, fluctuations in shear pattern (OSI) relative to baseline were apparent during all phases of the pressure cycle but did not differ significantly between the two postures.

Popliteal artery diameters were similar to those previously reported [5, 24] and while unaffected by INP, were greater during sitting than supine, most likely owing to the greater static intravascular pressure in this position [25]. At baseline, mean, anterograde, and retrograde shear rate decreased following the transition from the supine to a

sitting posture (Figure 2). This reduction in shear aligns with previous work investigating the effects of a sitting posture upon popliteal artery shear [26], and also some [27], but not all [8] investigations of the femoral artery during similar postures. The mechanisms for this reduction in shear rate during sitting remains to be fully elucidated, but probably relates, at least in part, to the haemodynamic influences of both local and systemic reflex-driven increases in vascular resistance [8, 23, 28].

The dynamic effects of intermittent negative pressure generated fluctuations in popliteal artery blood flow, shear rate and changes in shear pattern during both postures (Figure 2). The fluctuations in shear pattern were reflected by the OSI (Table I), which showed similar changes versus baseline during INP in both positions. Given the clinical benefits of INP upon disorders of lower limb circulation [2, 3, 29], and recent work investigating fluctuations in shear through the use of INP [15], resistance exercise [14] and also intermittent hypoxia [13], it is possible that fluctuation in shear rate forms an important, but only recently recognised stimulus for vascular function.

Despite the apparent changes in shear pattern found during both postures, the influence of INP specifically upon blood flow and shear rate was greater during a supine, than sitting position (Figure 4). While supine, blood flow and mean shear rate were significantly different to baseline values during all phases of interest. These were increased relative to baseline during the negative pressure (on) phase and immediately reduced when negative pressure transitioned back to the ambient atmosphere (off). In the sitting position however, both blood flow and mean shear rate only differed from baseline measures at the point of minimum shear, following the return from the negative pressure phase to atmospheric pressure. Interestingly, during the negative pressure phases the increase in mean shear rate was driven by a reduction in retrograde shear, in the absence of a significant effect upon the anterograde component. The converse then was apparent in the phase following a return to atmospheric pressure. These findings partly align with work investigating brachial artery responses to INP, where both the anterograde and retrograde components shifted in favour of greater forward shear during the negative pressure phase, while the opposite effect was observed during the return to atmospheric pressure [15], with a summative effect of a changing shear pattern at different phases of the INP cycle.

Under static conditions, the shear rate reductions that accompany postural change probably reflect reflex-driven increases in vascular resistance [23, 30, 31]. However, the additional effect of cyclic negative pressure changes during either posture may be more complex. Previous work has shown intravenous pressure to be readily influenced by external negative pressure, but only for a short period before recovering back to baseline (Figure 4; [17, 18]). This has the effect of transiently broadening the arteriovenous pressure difference [18] and probably accounts for the initial acceleration in blood flow normally observed during the initial cardiac cycles following the onset of negative pressure (Figure 4) [19, 20]. However, as external negative pressure transmits evenly to the tissue surrounding the blood vessels [16], transmural pressure, most importantly in the arteries and arterioles, is increased and rapidly recruits local non-baroceptor mediated mechanisms of vascular resistance above their resting levels [20, 22]. This leads to a subsequent deceleration in blood velocity, which if negative pressure remains constant, actually falls below baseline levels [20, 22]. In this study, while we observed the retrograde shear component to be reduced during the negative pressure phases, the converse was true upon the return to atmospheric pressure. Although speculative, given that these local resistance mechanisms are engaged during the negative pressure phase, when pressure returns to atmospheric levels, this increased resistance is likely to take a few moments to normalise, thus accounting for the greater retrograde shear component and reduced mean shear upon return to atmospheric conditions.

In the sitting position, INP had less influence upon shear rate than when supine. A number of factors may account for this, but the higher vascular resistance conferred by local, and baroreceptor-mediated vascular regulatory mechanisms following transition to the upright position is an attractive explanation [28, 30, 31]. Additionally, venous pressure is greater during sitting due to the additional haemostatic pressure elicited by gravity [32]. In the context of lower venous compliance at higher resting intravenous pressures [33], the additional volume that can be accommodated in the vessel in response to external pressure change may be attenuated in the upright position, reducing blood flow and thus shear.

Clinical context of INP-induced haemodynamic responses

While this study investigated healthy participants as a means of defining the haemodynamic effects of INP in health, the technique has emerged as a therapeutic intervention in vascular disease. Primarily, INP represents a mechanical adjunct in the treatment of peripheral arterial disease (PAD), with a growing body of work highlighting benefits within this group. The symptoms of PAD result from arterial lesions causing inadequate matching of tissue oxygen delivery versus demand. During periods of increased activity this can cause ischaemic claudication pain, or tissue loss in severe disease [34]. Ultimately, treatments that either increase tissue blood flow, oxygen delivery, or oxygen extraction have potential to reduce patient symptom burden.

As observed among our healthy participants, the immediate haemodynamic feature of INP is oscillations in local perfusion. Our study is the first to confirm this pattern in the large conduit arteries of the lower limb, however, similar patterns have been seen in the microcirculation and small foot arteries of clinical groups, including those with PAD [2, 3, 19], spinal injury [29], and chronic skin ulcers [35]. Indeed, these oscillations occur even in the presence

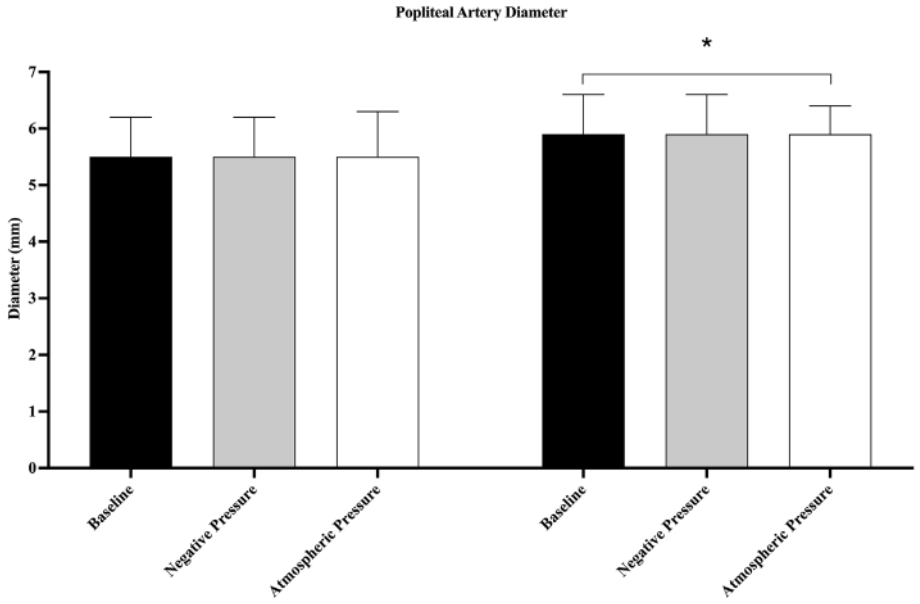

Figure 3. Popliteal artery diameter during supine and sitting postures. Popliteal artery diameter (mm) during each phase of the intermittent negative pressure cycle in the supine and sitting postures. Data are *mean ± SD.* *Significantly different from supine posture ($p<0.05$).

of significant arterial disease, such as patients with claudication symptoms (Fontaine class II [19, 36]) and those with ankle-brachial pulse indexes (ABI) ≤ 0.6 [35, 36, 37]. Cyclic changes in arteriovenous pressure gradient likely contribute to these oscillations, through periodic reductions in venous pressure augmenting local perfusion pressure (Figure 4). While these effects are visible here in healthy participants, they may become even more relevant during advanced disease where local resistance reflexes are blunted in order to prioritise tissue oxygen delivery in the face of reduced arterial inflow [38].

While the immediate effects of INP upon conduit artery flow can be clearly seen, persisting haemodynamic improvements have been found following longer treatment courses. Both Yagshyyev et al. [3] and Pettersen et al. [36] observed post-treatment improvements in skin perfusion and the myogenic component of vasomotion, respectively, following an extended treatment course in patients with PAD. Furthermore, improvements in ankle-brachial pulse index have been observed following treatments of 8 [35, 39] to 12 weeks [3]. Given the short daily "doses" involved with INP [37], it is likely that clinical benefits result from haemodynamic changes that persist beyond the immediate stimulus, rather than gains solely during the treatment interval itself. While increases in vasomotion may be one possible mechanism [36], the physiological importance of oscillating vascular shear stress is increasingly recognised for endothelial function [14, 15]. This effect has been proposed as the primary mechanism by which INP benefits patients with PAD or skin ulcers [34, 37]. Our results show clear oscillations in shear stress within the popliteal artery, adding support for shear changes as a potential mechanism by which INP exerts a clinical effect. Indeed, identical patterns of shear stress during INP have been shown to improve upper limb endothelial function [15], and such an improvement would positively impact both arterial and microvascular reactivity [40] with probable implications for both symptoms, function and wound healing among patients.

Relationships to exercise

At present, risk factor modification, optimised medical therapy, and supervised exercise interventions are the standard of care in PAD [36]. The earliest incarnations of INP were billed as 'passive vascular exercise' [41] and although direct haemodynamic comparisons between these two stimuli remain to be made, at least at a superficial level there are similarities in the haemodynamic pattern between exercise and INP (e.g. Figure 4). The additional benefit of INP may be in offering a passive intervention for those precluded from meaningful exercise due to their existing disease burden [3]. Crucially, INP has been shown to benefit patients with no-option critical limb ischaemia (Fontaine class III or IV), by reducing resting pain burden, in addition to the improvements in skin perfusion and ABI [3]. Congruently, increases in both pain-free and maximal walking distance have been demonstrated in patients with intermittent claudication (Fontaine class II)[2, 37, 39], although these improvements have not been found in all studies [42, 43]. It is notable however that the cumulative treatment dose was substantially lower in the latter studies owing to its non-home-based delivery, which may account for the lack of effect. Taken together these are important observations, as among patients too limited to undertake traditional exercise interventions, INP might provide a passive stimulus for symptom improvement that might later enable transition to more meaningful exercise activity.

Difference between disease phenotypes

While the bulk of existing work has focussed upon patients with PAD there is some clinical evidence for improved tissue healing among patients with arterial skin ulcers [35] and those following spinal injury [29]. As intermittent negative pressure differs substantially from traditional

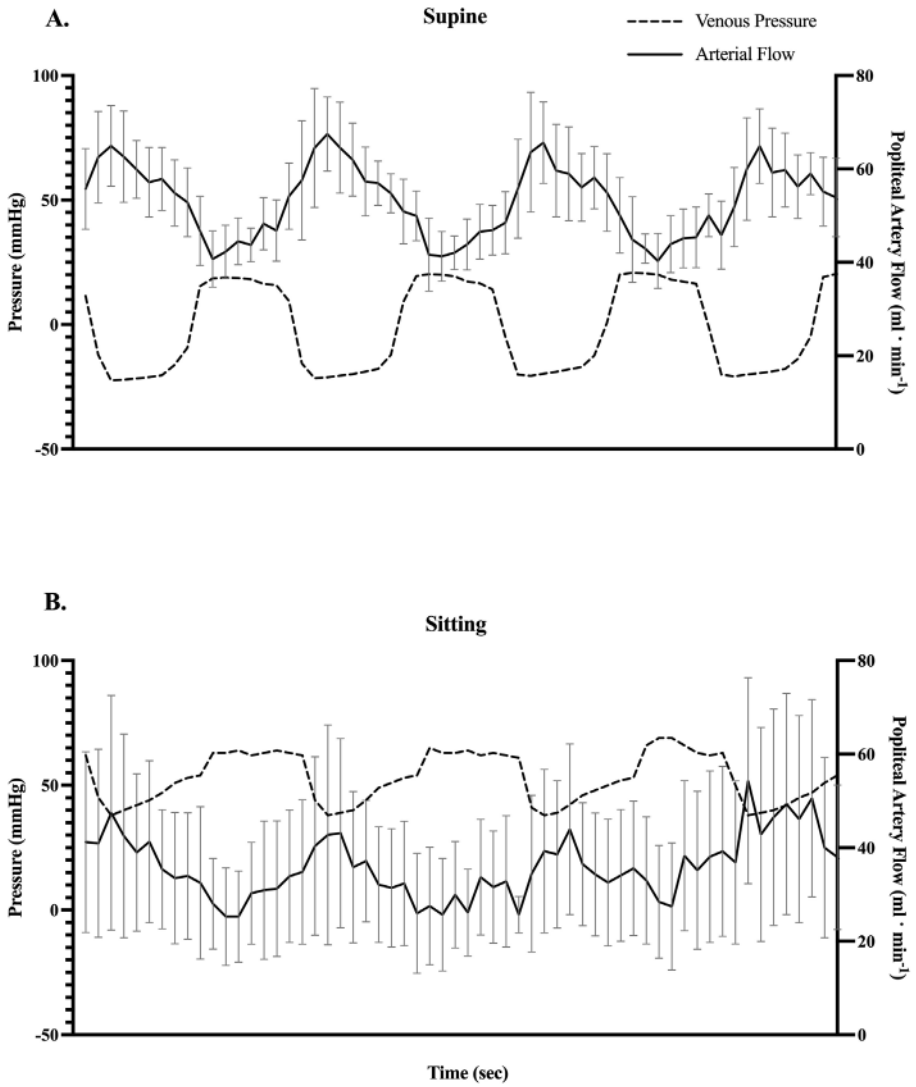

Figure 4. Popliteal artery blood flow and intravenous pressure during cycles of intermittent negative pressure. Popliteal artery flow (ml · min^{-1}; n=10) and a representative saphenous venous pressure trace (mmHg; n=1) during a series of negative pressure cycles in the supine (Panel A) and sitting (Panel B) positions. Negative pressure phases can clearly be seen in the venous pressure response. Note the reduced influence of negative pressure upon both intravenous pressure and popliteal artery flow during the sitting posture. Data for popliteal artery flow are *mean* ± *SD* (n=10) normalised to 1 second intervals. Saphenous venous pressure is included from a single representative participant, with a single value for each cardiac cycle. Solid line: Popliteal artery blood flow. Dashed line: Saphenous venous pressure at the level of the medial malleolus.

negative pressure wound therapy in the pattern, magnitude, and tissue volumes involved [44], it is possible that shear changes like those seen here during INP might improve conduit artery and microvascular reactivity [14, 15, 40] and improve regional blood flow. Additionally, although speculative, the fluctuations in microvascular and venous pressure could potentially influence fluid drainage from the region. This effect does however generate periodic increases in venous transmural pressure, and it would be reasonable to assume that such effects might be amplified in the case of venous insufficiency, due to greater reflux of venous blood during the negative pressure intervals. For this reason, further investigation of INP in venous disease is warranted.

Practical considerations for treatment

A key investigation within this study was the comparison of haemodynamic changes between postures. We found greater oscillation in blood flow and shear rate within the popliteal artery when INP was applied in supine, probably owing to less baseline regional vascular resistance in this position [8]. Although there are no defined thresholds for optimal shear stress, it is conceivable that greater blood flow and shear responses in the supine position could confer greater clinical benefit. Notwithstanding this finding, the actual shear oscillation occurring within cardiac cycles (OSI) showed similar changes regardless of posture. Given that previous clinical investigations delivered INP treatment in a sitting position, this may indicate that changes in OSI represent the primary stimulus to positive vascular change, rather than changes in the magnitude of shear rate [45]. Regardless, future work is required to investigate any clinically relevant differences between INP use in sitting or supine postures, particularly given the ease of home use and patient acceptability when used in the seated position [46].

Limitations

We acknowledge that our participants comprised a group of health young adults whose responses to INP may differ from clinical populations. The decision to study a healthy cohort of individuals was twofold; first, understanding the influence of INP upon normal, intact physiology provides better direction as to the specific mechanisms that should be investigated in a larger trial among clinical groups. Second, as INP is anticipated to modulate local vascular resistance mechanisms to at least some degree, given the very small changes that would typically be anticipated, it was important that extraneous error within the data was limited as much as possible to allow detection of physiologically important changes. Regardless of the above, previous work examining skin blood flux during INP appears similar both in healthy participants [20] and in those with peripheral arterial disease [19, 34] suggesting the underlying mechanisms may remain broadly similar regardless of the population being studied.

Our lack of investigation of endothelial function in this study is a limitation to untangling the separate effects of shear rate and shear pattern in the lower limb. Although we observed changes in conduit artery shear rate and pattern in response to INP, we cannot conclude whether the influence upon markers of endothelial function, such as flow mediated dilatation are similarly influenced. We encourage future work to extend these findings.

Finally, it is possible that the action of the negative pressure chamber could generate small oscillations in angle between the ultrasound probe and vessel. Given the potential impact upon velocity measurements, care was taken to ensure the probe position was maintained constant during all phases of testing. Furthermore, the pattern of change in blood flow at the popliteal artery largely mirrors that of skin blood flux seen during pilot testing, suggesting our results to represent a true effect of negative pressure upon blood velocity.

Conclusions

Intermittent negative pressure induced changes in shear rate, and fluctuations in the pattern of shear in the popliteal artery. The influence of INP upon the retrograde component appeared to account for much of these changes. Moreover, the influence of INP upon shear rate was greater during a supine than sitting posture, but fluctuation in shear rate was similar regardless of position. Given the positive clinical effects of INP despite the lower shear rates during the upright posture, the fluctuations in shear rate may be a key mechanistic factor underlying the clinical effect of this novel therapy. Furthermore, we highlight a variability in response to INP depending on posture, the clinical relevance of which is currently unclear. Future work should aim to investigate the effects of INP upon shear rate and endothelial function among those with vascular disease, with specific emphasis upon the pattern of shear elicited by negative pressure, and any interaction of these with posture.

Electronic supplementary material

The following electronic supplementary material is available with this article at https://doi.org/10.1024/0301-1526/a001172

ESM 1. Systemic circulatory variables at key points during the intermittent negative pressure cycle during supine and sitting postures. Supplementary information in addition to Figure 1 (Table).

References

1. Kim MS, Hwang J, Yon DK, Lee SW, Jung SY, Park S, et al. Global burden of peripheral artery disease and its risk factors, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet Glob Health. 2023;11(10):e1553–65.
2. Hoel H, Pettersen EM, Høiseth LØ, Mathiesen I, Seternes A, Hisdal J. A randomized controlled trial of treatment with intermittent negative pressure for intermittent claudication. J Vasc Surg. 2021;73(5):1750–1758.e1.
3. Yagshyyev S, Hausmann P, Li Y, Kempf J, Zetzmann K, Dessi K, et al. Intermittent negative pressure therapy in patients with no-option chronic limb-threatening ischemia. Vasa. 2023;52:402–8.
4. Delis K, Labropoulos N, Nicolaides A, Glenville B, Stansby G. Effect of Intermittent Pneumatic Foot Compression on Popliteal Artery Haemodynamics. Eur J Vasc Endovasc Surg. 2000;19:270–7.
5. Sheldon RD, Roseguini BT, Thyfault JP, Crist BD, Laughlin MH, Newcomer SC. Acute impact of intermittent pneumatic leg compression frequency on limb hemodynamics, vascular function, and skeletal muscle gene expression in humans. J Appl Physiol. 2012;112:2099–109.
6. Padilla J, Jenkins NT, Laughlin MH, Fadel PJ. Blood pressure regulation VIII: resistance vessel tone and implications for a pro-atherogenic conduit artery endothelial cell phenotype. Eur J Appl Physiol. 2014;114:531–44.
7. Tremblay JC. Through thick and thin: The interdependence of blood viscosity, shear stress and endothelial function. Exp Physiol. 2020;105:232–3.
8. Newcomer SC, Sauder CL, Kuipers NT, Laughlin MH, Ray CA. Effects of posture on shear rates in human brachial and superficial femoral arteries. Am J Physiol-Heart Circ Physiol. 2008;294:H1833–9.
9. Casey DP, Padilla J, Joyner MJ. α-Adrenergic Vasoconstriction Contributes to the Age-Related Increase in Conduit Artery Retrograde and Oscillatory Shear. Hypertension. 2012;60(4):1016–22.
10. Green DJ, Hopman MTE, Padilla J, Laughlin MH, Thijssen DHJ. Vascular Adaptation to Exercise in Humans: Role of Hemodynamic Stimuli. Physiol Rev. 2017;97:495–528.
11. Naylor LH, Carter H, FitzSimons MG, Cable NT, Thijssen DHJ, Green DJ. Repeated increases in blood flow, independent of exercise, enhance conduit artery vasodilator function in humans. Am J Physiol-Heart Circ Physiol. 2011;300:H664–9.
12. Thijssen DHJ, Dawson EA, Tinken TM, Cable NT, Green DJ. Retrograde Flow and Shear Rate Acutely Impair Endothelial Function in Humans. Hypertension. 2009;53:986–92.
13. Hanson BE, Iwamoto E, Mouser BL, Miller KA, Casey DP. Hypoxia offsets the decline in brachial artery flow-mediated

dilation after acute inactivity. Am J Physiol-Regul Integr Comp Physiol. 2022;323:R787–96.
14. Hanson BE, Casey DP. Intermittent versus continuous handgrip exercise and peripheral endothelial function: impact of shear rate fluctuations. J Appl Physiol. 2023;135:892–901.
15. Holder SM, Dawson EA, Brislane Á, Hisdal J, Green DJ, Thijssen DHJ. Fluctuation in shear rate, with unaltered mean shear rate, improves brachial artery flow-mediated dilation in healthy, young men. J Appl Physiol. 2019;126:1687–93.
16. Olsen H, Länne T. Reduced venous compliance in lower limbs of aging humans and its importance for capacitance function. Am J Physiol-Heart Circ Physiol. 1998;275(3):H878–86.
17. Caro CG, Foley TH, Sudlow MF. Early effects of abrupt reduction of local pressure on the forearm and its circulation. J Physiol. 1968;194:645–58.
18. Lott MEJ, Herr MD, Sinoway LI. Effects of transmural pressure on brachial artery mean blood velocity dynamics in humans. J Appl Physiol. 2002;93:2137–46.
19. Hoel H, Høiseth LØ, Sandbæk G, Sundhagen JO, Mathiesen I, Hisdal J. The acute effects of different levels of intermittent negative pressure on peripheral circulation in patients with peripheral artery disease. Physiol Rep. 2019;7:e14241. https://doi.org/10.14814/phy2.14241
20. Sundby ØH, Høiseth LØ, Mathiesen I, Jørgensen JJ, Weedon-Fekjaer H, Hisdal J. Application of intermittent negative pressure on the lower extremity and its effect on macro- and microcirculation in the foot of healthy volunteers. Physiol Rep. 2016;4(17):e12911.
21. Eiken O, Kolegard R. Comparison of vascular distensibility in the upper and lower extremity. Acta Physiol Scand. 2004;181:281–7.
22. Lott MEJ, Hogeman C, Herr M, Bhagat M, Kunselman A, Sinoway LI. Vasoconstrictor responses in the upper and lower limbs to increases in transmural pressure. J Appl Physiol. 2009;106:302–10.
23. Padilla J, Young CN, Simmons GH, Deo SH, Newcomer SC, Sullivan JP, et al. Increased muscle sympathetic nerve activity acutely alters conduit artery shear rate patterns. Am J Physiol-Heart Circ Physiol. 2010;298:H1128–35.
24. Parker BA, Ridout SJ, Proctor DN. Age and flow-mediated dilation: a comparison of dilatory responsiveness in the brachial and popliteal arteries. Am J Physiol-Heart Circ Physiol. 2006 Dec;291(6):H3043–9.
25. Malhotra A, Cohen D, Syms C, Townsend RR. Blood Pressure Changes in the Leg on Standing. J Clin Hypertens. 2002;4:350–4.
26. Restaino RM, Holwerda SW, Credeur DP, Fadel PJ, Padilla J. Impact of prolonged sitting on lower and upper limb micro- and macrovascular dilator function. Exp Physiol. 2015;100:829–38.
27. Trinity JD, Groot HJ, Layec G, Rossman MJ, Ives SJ, Richardson RS. Impact of Age and Body Position on the Contribution of Nitric Oxide to Femoral Artery Shear Rate: Implications for Atherosclerosis. Hypertension. 2014;63:1019–25.
28. Vissing SF, Secher NH, Victor RG. Mechanisms of cutaneous vasoconstriction during upright posture. Acta Physiol Scand. 1997;159:131–8.
29. Sundby ØH, Irgens I, Høiseth LØ, Mathiesen I, Lundgaard E, Haugland H, et al. Intermittent mild negative pressure applied to the lower limb in patients with spinal cord injury and chronic lower limb ulcers: a crossover pilot study. Spinal Cord. 2018;56:372–81.
30. Groothuis JT, Thijssen DHJ, Kooijman M, Paulus R, Hopman MTE. Attenuated peripheral vasoconstriction during an orthostatic challenge in older men. Age Ageing. 2008;37:680–4.
31. Smit AAJ, Halliwill JR, Low PA, Wieling W. Pathophysiological basis of orthostatic hypotension in autonomic failure. J Physiol. 1999;519(1):1–10.
32. Pollack AA, Wood EH. Venous Pressure in the Saphenous Vein at the Ankle in Man during Exercise and Changes in Posture. J Appl Physiol. 1949;1:649–62.
33. Halliwill JR, Minson CT, Joyner MJ. Measurement of limb venous compliance in humans: technical considerations and physiological findings. J Appl Physiol. 1999;87:1555–63.
34. Sundby ØH, Høiseth LØ, Mathiesen I, Weedon-Fekjær H, Sundhagen JO, Hisdal J. The acute effects of lower limb intermittent negative pressure on foot macro- and microcirculation in patients with peripheral arterial disease. Kirchmair R, editor. PLOS ONE. 2017;12(6):e0179001.
35. Sundby ØH, Høiseth LØ, Mathiesen I, Jørgensen JJ, Sundhagen JO, Hisdal J. The effects of intermittent negative pressure on the lower extremities' peripheral circulation and wound healing in four patients with lower limb ischemia and hard-to-heal leg ulcers: a case report. Physiol Rep. 2016;4(20):e12998.
36. Pettersen EM, Hoel H, Torp H, Hisdal J, Seternes A. The Effect of 12-Week Treatment with Intermittent Negative Pressure on Blood Flow Velocity and Flowmotion, Measured with a Novel Doppler Device (Earlybird). Secondary Outcomes from a Randomized Sham-Controlled Trial in Patients with Peripheral Arterial Disease. Ann Vasc Surg. 2022;86:144–57.
37. Hoel H, Pettersen EM, Høiseth LØ, Mathiesen I, Seternes A, Hisdal J. Lower Extremity Intermittent Negative Pressure for Intermittent Claudication. Follow-Up after 24 Weeks of Treatment. Ann Vasc Surg. 2021;75:253–8.
38. Stranden E. Dynamic recording of vasoconstrictor response to increased vascular transmural pressure. A study in patients with lower limb atherosclerosis. Acta Chir Scand. 1984;150(1):25–30.
39. Himmelstrup H, Himmelstrup B, Mehlsen J, Trap-Jensen J. Effects of Vacusac in intermittent claudication: a controlled cross-over study. Clin Physiol. 1991;11:263–9.
40. Santamaría R, González-Álvarez M, Delgado R, Esteban S, Arroyo AG. Remodeling of the Microvasculature: May the Blood Flow Be With You. Front Physiol. 2020;11:586852.
41. Herrmann LG, Reid MR. The conservative treatment of arteriosclerotic peripheral vascular diseases: Passive vascular exercises (PAVEX Therapy). Ann Surg. 1934;100:750–60.
42. Hageman D, Fokkenrood HJP, Van Deursen BAC, Gommans LNM, Cancrinus E, Scheltinga MRM, et al. Randomized controlled trial of vacuum therapy for intermittent claudication. J Vasc Surg. 2020;71:1692–1701.e1.
43. Afzelius P, Molsted S, Tarnow L. Intermittent vacuum treatment with VacuMed does not improve peripheral artery disease or walking capacity in patients with intermittent claudication. Scand J Clin Lab Invest. 2018;78:456–63.
44. Dumville JC, Land L, Evans D, Peinemann F. Negative pressure wound therapy for treating leg ulcers. Cochrane Wounds Group, editor. Cochrane Database Syst Rev [Internet]. 2015 Jul 14 [cited 2024 Oct 22];2016(12). https://doi.org/10.1002/14651858.CD011354.pub2
45. Green DJ, Carter HH, Fitzsimons MG, Cable NT, Thijssen DHJ, Naylor LH. Obligatory role of hyperaemia and shear stress in microvascular adaptation to repeated heating in humans. J Physiol. 2010;588:1571–7
46. Sedgwick CE, Growcott C, Akhtar S, Parker D, Pettersen EM, Hashmi F, et al. Patient and clinician experiences and opinions of the use of a novel home use medical device in the treatment of peripheral vascular disease – a qualitative study. J Foot Ankle Res. 2021;14(1):61.

History
Submitted March 14, 2024
Accepted after revision November 29, 2024
Published online January 9, 2025

Acknowledgments
We wish to thank our participants for giving their time to contribute to the study.

Conflict of interest
NC is employed as a PhD student by Otivio AS with match-funding from the Norwegian Research Council through the Industrial Ph.D.

Scheme/Public Sector Ph.D. Scheme, Ref. 329428. IM is the primary shareholder and chief scientific officer of Otivio AS who manufacture the intermittent negative pressure device used in this study.

Author contributions

NAC, IM and JH conceived and designed the research. NAC, JH and LØH performed experiments. NAC undertook data analysis. NAC drafted the manuscript. NAC, LØH, IM and JH edited, revised, and approved the final manuscript. All listed authors assume responsibility for the work.

Funding

This study was funded by Otivio AS and the Norwegian Research Council, Grant Ref: 329428.

ORCID

Nigel A. Callender
https://orcid.org/0000-0001-9658-5266
Lars Øivind Høiseth
https://orcid.org/0000-0003-0669-8297
Iacob Mathiesen
https://orcid.org/0000-0003-0397-0742
Jonny Hisdal
https://orcid.org/0000-0002-0224-7854

Correspondence address

Nigel A. Callender
Faculty of Medicine
University of Oslo
Klaus Torgårds vei 3
0372 Oslo
Norway

nigelac@uio.no