Low-pressure sequential compression of lower limbs enhances forearm skin blood flow

Abstract

Purpose: We investigated whether forearm skin blood flow could be improved when a multilayer pulsatile inflatable suit was applied at a low pressure to the lower limbs and abdomen. We hypothesized that a non-invasive purely mechanical stimulation of the lower limbs could induce remote forearm blood flow modifications.

Methods: The pulsatile suit induced a sequential compartmentalized low compression (65 mmHg), which was synchronized with each diastole of the cardiac cycle with each phase evolving centripetally (lower limbs to abdomen). Modifications of the forearm skin blood flow were continuously recorded by laser Doppler flowmetry (LDF) at baseline and during the pulsatile suit application. Endothelium-dependent and endothelium-independent vasodilations of the forearm skin microcirculation were measured by LDF in response to a local transdermal iontophoretic application of acetylcholine (ACh-test) and to hyperthermia (hyperT-test).

Results: Twenty-four healthy volunteers, 12 men and 12 women (43±14 years) were included in the study. LDF responses increased 1) under pulsatile suit (97±106%, p<0.01), 2) during ACh-test (338±336%, p<0.001) and 3) during hyperT-test (587±383%, p<0.001). No significant gender differences were seen. There was a significant linear positive regression between LDF response under pulsatile suit and during ACh-test (R= 0.79, p<0.001) and during hyperT-test (R=0.62, p=0.004). No adverse effects were reported.

Conclusions: This proof-of-concept study shows that a sequential low-pressure compression applied to the lower limbs and abdomen induces a significant increase of the forearm skin blood flow, suggestive a systemic effect. Mechanisms still need to be investigated.
Vasomotor regulation is mediated by local mechanical factors, which induce the release of various circulating vasoactive molecules [1], and by the autonomic nervous system. The endothelium exerts a pivotal role to ensure this regulation at the level of conduit and resistant arteries [2]. The enhancement of the longitudinal shear forces applied on the endothelium (shear stress) is probably one of the most powerful local mechanical factors that stimulate the activity of the endothelial nitric oxide synthase (eNOS) resulting in an increase in the arterial inner diameter [3,4]. The endothelial release of nitric oxide (NO) induces the relaxation of vascular smooth muscle cells and the decrease of vascular tone [5].

Shear stress is directly proportional to the blood flow velocity, and inversely proportional to the 3rd power of the arterial radius \((\tau=\frac{4\mu Q}{\pi R^3}\) where \(\tau\) is the shear stress, \(Q\) is the blood flow volume, \(R\) is the arterial radius and \(\mu\) is the viscosity) [4,6]. When the arterial radius increases, the shear stress tends to normalize. An increase in shear stress, induced by high compression of blood vessels during cuff inflation, also triggers the endothelium-depndant flow-mediated vasodilation (FMD) observed in downstream arterial beds [7,8,9].

The purpose of the present study was to assess the ability of a low-pressure pulsatile inflatable suit, applied to the lower limbs and the abdomen, to induce non-invasively and by a pure mechanical effect, remote forearm blood flow modifications.

Methods

Subjects

The study was conducted in accordance with the Helsinki Declaration, the Good Clinical Practices, the European Directive 93/42/EC amended by 2007/47/EC and the French Laws and Regulations (Decree n° 2006/477 of 26 April 2006, Order of 24 August 2006 related to 2004-806 of 9 August 2004 on biomedical research). The study protocol and its appendices (including the information to the subjects and the consent form) received authorization from the “Comité de Protection des Personnes CPP IDF VI” – Pitié Salpêtrière, 47, Boulevard de l’Hôpital 75651 Paris Cedex 13 France (N° 88-13). Healthy subjects (n=24; 12 men, 12 women) aged 18 to less than 65 years were included after their informed consents were obtained. Non-inclusion criteria were on-going venous thrombosis, pregnant women, smokers, current medication and subjects participating to another clinical study. All subjects were volunteers and no incentives, financial compensation or gifts were provided by the investigators.

Protocol

Subjects were successively submitted to 1) a pulsatile suit session, 2) an acetylcholine iontophoresis (ACh-test) and 3) a local cutaneous hyperthermia (hyperT-test) (Figure 1). ACh-test and hyperT-test, the two classical reference methods for the evaluation of endothelium-dependent and endothelium-independent FMD in the forearm skin microcirculation [10, 11], were performed locally on the right forearm. Modifications of the forearm skin blood flow were recorded by laser Doppler flowmetry (LDF). All acquisitions were conducted in a room at a constant ambient temperature of 19°C. Systolic, diastolic and mean blood pressures and heart rate were noted at baseline and at the end of the pulsatile inflated suit procedure. A return to baseline of the LDF values was required before moving to the next session. For each subject, the entire study duration was 3 hours (Figure 1).

Laser Doppler Flowmetry

The LDF (Periflux System 5000®, Perimed, Järfälla, Sweden) continuously recorded cutaneous blood flow, expressed in arbitrary units, with an optical sensor on the subject’s right forearm, 4 cm below the antecubital fossa. The sensor was maintained at the same location for the duration of the study with self-adhesive tape. The temperature of the sensor was stabilized at 32°C throughout the duration of the recording, except during the six minutes of the hyperT-test. The changes in LDF reflect the modifications in microcirculation; the LDF sensor detects the frequency of the oscillation induced by the Doppler frequency shift of the red blood cells in the skin vasculature and translates the frequency to an intensity oscillation [12]. The LDF signal was continuously transferred to a PC computer equipped with the Perisoft 2.55 Software (Perimed, Järfälla, Sweden). The maximal shift of the LDF signal was noted as the peak value; the blood flow velocities modifications were expressed as percentage: \([\text{peak value} – \text{baseline value}] / \text{baseline value}\) × 100.

Pulsatile suit

The pulsatile suit session was performed using an inflatable suit device (Stendo® Society, Louviers France and Stendo® software) composed of two subunits: 1) an electro-pneumatic console; and, 2) a multilayer, inflatable “clothing” item, equipped with one air tube for each lower limb, connected to the console (Figure 2). The subject was in a supine position. The operator placed five ECG electrodes on his chest and a blood pressure cuff. The suit was then closed on the legs thighs and the abdomen of the subject, starting at the lower extremity of the
body, using the self-adhesive straps in order to tailor it as much as possible to the measurements of the subject without the suit being too tight. All of the connections between the subject and the console were checked and the settings for the session were entered in the console: 20 minutes for the session duration and 65 mmHg for the inflation pressure. The pulsatile suit induced a sequential compartmentalized compression/decompression evolving centripetally (lower limbs to abdomen), synchronized with the phases of the cardiac cycle determined from the electrocardiogram signal. Diastole was the reference signal for the launch of inflation and systole was the reference signal for the launch of deflation. After the return to baseline of the LDF values, preparation for the ACh-test session lasted for about 10 min.

Although no adverse event was expected, patients were systematically asked if they experienced discomfort, pain or skin injury particularly during the 3-hour duration of the study.

**Acetylcholine test (ACh-test)**

In addition to the Perimed device, the ACh-test requires one active (PF383®) and one passive (PF384®) electrodes (Perimed, Craponne, France) both connected to a battery-powered iontophoresis controller (PeriIont328, Perimed, Järfalla, Sweden). PF383®, which is a drug delivery electrode, includes a foam adhesive ring impregnated with an ACh solution of 20 mg/ml in deionized water. This concentration initiates the cutaneous FMD [10]. The design of the active PF383® electrode allowed the record sensor to be positioned at its

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**FIGURE 1.** Study design. Various steps and duration of the protocol. LDF: laser Doppler flowmetry, Ach: acetylcholine, T°: hyperthermia

**FIGURE 2.** Description of the pulsatile suit system. A: multilayer inflatable suit; B: electro-pneumatic console; C: subject in a supine position with the pulsatile suit; D: schematic description of the compression/decompression induced by the pulsatile suit.
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Individual baseline characteristics of the 24 participants. The mean values with their standard deviations are shown in the last two lines of the table.

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; M, men; W, women; SD, standard deviation.
center. The passive electrode was located at a 2-cm distance from the active one, and allowing creating an electric current (0.1 mA, duration 10s). This current applied between both electrodes induced the ACh iontophoresis through the skin, towards the subcutaneous microvasculature. Three stimulations were performed successively separated by a 120-s interval, before LDF measurements. The ACh-test therefore lasted no more than 6 min. Then after the return to baseline of the LDF values, preparation for the hyper-T-test session lasted for about 10 min.

**Hyper-T-test (hyperT-test)**

The active LDF electrode with heating capacity, Probe 475° (Perimed, Järfälla, Sweden) was set to 44° C throughout the duration of the test (6 min). At 6 min, the hyperT-test was stopped since LDF values were very high in order to limit the total duration of the procedure (pulsatile suit + ACh-test + hyperT-test). The response of skin blood flow to local hyperT-test is usually biphasic with an initial rapid peak and a second slower phase [13]. The duration of the hyperT-test allowed us to measure the phase 1 (initial rapid peak) of this response.

**Statistical analysis**

Results are expressed as mean ± standard deviation (Min, Max) and as [(peak value-baseline value)/baseline value] x 100 for the LDF modifications (expressed as percentage ± SD) in each three conditions (pulsatile suit application, ACh-test and hyperT-test). ANOVA for repeated measurements with gender as grouping variable was performed to compare blood pressures, heart rates and LDF modifications absolute values as well as absolute values of difference between base and peak LDF signal in each condition. As no gender difference was observed, the three different conditions were compared using an ANOVA for repeated measurements without grouping variable with post hoc Bonferroni corrected and Student’s Newman-Keuls test.

Linear regression analyses were used to evaluate the relationship between the modifications of the LDF signal (delta signal: peak value – basal value) recorded in each of the three conditions and age, and between pulsatile inflated suit and ACh-test or hyperT-test (MedCalc Software, Mariakerke, Belgium). Differences with a p value <0.05 were considered significant.

**Results**

**Baseline characteristics and safety**

Baseline characteristics of the volunteers are shown in Table 1. We found no abnormalities on skin examinations and the subjects reported no adverse effects.

**Timing of the different phases of the protocol**

Table 2. For the hyperT-test, the return to the baseline values of LDF was not reached until the end of the study for 10 subjects. For the other 14 subjects, this return occurred at 18±6 min.

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LDF, laser Doppler flowmetry; ACh-test, acetylcholine iontophoresis; hyperT-test, local cutaneous hyperthermia.

<table>
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<th>Duration of stimulation (minutes)</th>
<th>Time from start of stimulation to peak of LDF (minutes)</th>
<th>Time from cessation of stimulation to the return of LDF baseline values (minutes)</th>
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**TABLE 2. Timing of the different phases of the protocol**

LDF values increased during the pulsatile suit session by 97±106% (p<0.01), indicative of a two-fold increase of the forearm skin microcirculation blood flow; i.e., at a distant site from where the pulsatile suit was applied (Figure 3) without significant difference between females and males (149±128% vs. 45±36%, respectively, NS). LDF response to the pulsatile
inflated suit was negatively correlated with age (R=0.48, p=0.027). At the end of the pulsatile suit session, systolic, diastolic and mean blood pressures had slightly but significantly decreased compared with the values prior to stimulation: 120±10 to 115±10 (p<0.001), 76±7 to 74±7 (p<0.001) and 90±9 to 87±8 (p<0.001) mmHg, respectively. Heart rate was not significantly modified at the end of the procedure (64±6 bpm before vs. 63±7 bpm at the end).

Increase in LDF signal during ACh-test and hyperT-test were 338±336 % (p<0.001) and 587±383% (p<0.001), respectively (Figure 3). LDF response during hyperT-test was significantly higher compared with the two other conditions (p<0.001). There was no significant gender difference in LDF response during ACh-test (323±342% vs. 353±345%, males vs. females) or during hyperT-test (531±280% vs. 654±487%, males vs. females). As expected, the endothelium-dependent vasodilation (ACh-test) was negatively correlated with the age (R = 0.42, p = 0.042), but the maximal endothelium-independent vasodilation (hyperT-test) was not significantly correlated with age.

A significant linear relationship was found between LDF response during pulsatile inflated suit device and ACh-test (R=0.79, p<0.001, Figure 4) and, to a lesser extent, hyperT-test (R=0.62, p=0.007, Figure 5).

**Discussion**

In this study, we showed for the first time, in a small cohort of healthy volunteers, that lower limbs and abdomen sequential compression with a pulsatile suit at 65 mmHg for 20 minutes, induced a statistically significant 2-fold elevation of blood flow at the skin forearm with good tolerability and safety.

Several studies have investigated the consequences of acute enhanced external counter pulsation (EECP) on blood flow changes in distant vascular territories [14-18]. Cuffs were inflated at a high pressure (up to 300 mmHg), which greatly altered arterial physiological hemodynamics [19], blood flow was retrograde and turbulent in the femoral artery but anterograde and laminar in the brachial artery [20]. Nevertheless, Gurovich et al. [21] have shown, in a study that included 18 young men (25±4 years), that a single 45-min session of EECP with a pressure of 250 mmHg increased FMD in the femoral and brachial arteries compared with baseline, despite different blood flow patterns (retrograde-turbulent blood flow in the femoral artery and anterograde-laminar blood flow in the brachial artery). Both flow patterns created acute increases in endothelial shear stress. In the same study, 45-min session of EECP at 50 mmHg did not show any improvement of FMD.

![FIGURE 3. Percentage of increase in forearm cutaneous microvascular circulation after each type of stimulus: pulsatile suit, acetycholine iontophoresis and hyperthermia. The increases in LDF signal were higher during acetycholine iontophoresis and hyperthermia compared to pulsatile suit application (*** p < 0.001)](image)

![FIGURE 4. Correlation between the increase in laser Doppler flow during pulsatile suit stimulation and the acetycholine iontophoresis test. Significant linear relationship of LDF response between the pulsatile suit and the ACh iontophoresis test (p<0.001). Delta signal: peak value – basal value, AU: arbitrary unit.)](image)
The duration and the number of the sessions were variable from one study to another. Beck et al. [20], described EECP duration of thirty-five 1-hour sessions (5 days a week for 7 weeks) with inflation pressures of 300 mmHg, in patients with ischemic left ventricular dysfunction, and showed improvement of brachial and femoral artery endothelial function. The pulsatile inflatable suit in our study was used with cuffs inflated at a value below the diastolic blood pressure. No studies have been reported using pressures of 65 mmHg and with a single session of duration comparable to ours.

Measurement of blood flow changes in the skin microcirculation using LDF coupled with ACh iontophoresis represents a reliable noninvasive method for the assessment of endothelial function and was found to be correlated to the FMD of the brachial artery, as has been previously demonstrated by our group [10].

The order of the sessions was not randomized because the duration of the return to the baseline of the LDF values could lead to a wrong interpretation of LDF values observed during the pulsatile suit session if the latter occurred after ACh-test or especially after hyperT-test.

Several physiological mechanisms can be discussed to explain the remote effect of the pulsatile suit on the cutaneous blood flow. We speculate that the tangential force derived by the friction of the circulating blood at the endothelial surface at the site of compression increases downstream shear stress [2, 4, 22, 23], which, in turn, induces vasodilation. This action could stimulate the release of factors that induce a systemic effect with an increase in skin microcirculation such as NO [24]. The production of NO is regulated by eNOS, and eNOS transcription is enhanced by shear stress. It has been shown that the half-life of eNOS mRNA is as long as 10 to 35 hours [25]. NO production is induced by shear stress in two phases: the first one is a brief and transient NO burst that is followed by a second sustained phase of low NO production. The second sustained phase can last as long as shear stress is imposed [25]. Many other mechanisms may be involved during this period, and several vasoactive peptides are released by endothelial cells in response to shear stress induced by blood flow increase, such as prostacyclin, adenosine, C-type natriuretic peptide (CNP), vasoactive intestinal peptide (VIP) and adrenomedullin. Moreover, these vasoactive peptides may interact with each other and enhance the vasorelaxation: VIP produces an endothelium-dependent relaxation by releasing NO and/or prostacyclin [25], and there is an important reciprocal regulation of vascular tone by natriuretic peptides (CNP in particular) and NO [26]. We hypothesize that the shear stress induced by the pulsatile suit could lead to the release of all these vasoactive peptides, resulting in cumulative and delayed vasodilation.

Our study was a non-invasive feasibility study in healthy subjects and, therefore, did not allow us to evaluate possible mechanisms via blood or urine tests or via measurements such as shear stress, brachial artery blood flow or common carotid artery blood flow measured by Doppler ultrasound.

We have shown that the pulsatile inflated suit is a reliable method to non-invasively improve endothelial function. ACh-test is the reference test to assess the endothelial function and NO dependent vasodilation [10]. The initial rapid peak of skin blood flow induced by hyperT-test, which we recorded in our study, was indicative of the NO-independent skin vasodilation [13]. Notably, there was a significant linear relationship between LDF response with ACh test and pulsatile suit device (R=0.79 P<0.001) as well as between hyperT-test and pulsatile device (R=0.62, p=0.004). This suggests that the low pressure pulsatile suit was able to induce an increase in blood flow in distant vascular territories, both via endothelial- and non-endothelial-dependent vasodilation.

Another physiological hypothesis could be the mobilization of a fraction of the venous blood volume from the lower limbs resulting in a transient increase in the venous blood flow return to the heart and an extended increase in the...
blood volume in the supradiaphragmatic areas. Heart rate was not significantly altered at the end of the pulsatile suit session, whereas blood pressure significantly decreased. We did not measure cardiac output so we cannot assume that the drop in blood pressure observed with a stable heart rate was the consequence of a decrease in peripheral vascular resistances (i.e., peripheral vasodilation).

In our work, we observed a large gender difference in mean percentage increase in LDF at the end of pulsatile suit session. Nevertheless, this difference was not statistically significant (p=0.068). This was essentially related to the two youngest male subjects (21 and 26 years) who presented high basal values and very high values of LDF during pulsatile suit session, shifting upwards these values. They also presented a high percentage increase in LDF under acetylcholine stimulation as well as under hyperthermia. Exclusion of these two individuals lowers the difference (88±42% in men vs. 45±36% in women) of course without any statistical difference. According to Dixon’s criteria, we could not reject any of the outliers.

Potential clinical applications of this research include the management of diseases associated with endothelial dysfunction, leading, in particular, to impaired vasodilation. Such conditions include peripheral arterial disease, hypertension, coronary artery disease, chronic heart failure, diabetes, chronic renal failure [27] and erectile dysfunction. As long as skin microcirculation is affected by the action of the pulsatile suit as shown in our study, it is also reasonable to consider the use of this technique in other situations, such as microsurgical reconstruction of skin defects.

Conclusions

This noninvasive study demonstrated that a 20-min session of 65 mmHg pressure sequential compression of the lower limbs and the lower part of the abdomen, synchronized with each diastole, allowed a significant increase in the blood flow of the forearm skin micrcirculation, without any adverse effect. This study paves the way for clinical investigations in conditions where endothelial and microcirculatory dysfunction may require improved local and systemic microcirculatory flow.

Acknowledgements

The authors thank the STENDO Society (17, rue du port 27400 Louviers, France) who graciously lent the non-invasive pulsatile device, Michele Pauty who performed the laser Doppler procedures and Dominique Delmas for her fruitful discussion and technical support.

Abbreviations

- ACh acetylcholine
- ANOVA analysis of variance
- ECG electrocardiogram
- EECP enhanced external counter pulsation
- LDF Flow Mediated Vasodilation
- FMD

References


