The use of microcirculatory techniques in the assessment of pathophysiology, diagnosis and management of critical limb ischemia

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Chapter 8

Postural changes in capillary pressure in the hallux of healthy volunteers

Jurgen C. de Graaff, Dirk Th. Ubbink, Sjoerd M. Lagarde, Michael J.H.M. Jacobs

Abstract

Background Capillary circulation is delicately regulated by microvascular constriction mechanisms, thereby controlling capillary perfusion and transmural pressure. The influence of posture on capillary flow has been investigated both in diseased and healthy people. However, its influence on capillary pressure is less investigated. Hence, we measured capillary pressure for the first time dynamically in the supine and sitting positions in the hallux of healthy volunteers.

Methods The capillaries in the eponychium of the hallux were punctured using a micropipette connected to a micropressure system (900A, WPI). Also, non-invasive peripheral arterial and invasive venous pressures were measured in both positions.

Results The rise in systolic capillary pressure from supine to sitting position (32 mm Hg; from 39 to 71 mm Hg, resp.) was significantly (p<.001) smaller than the rise in systolic arterial toe pressure (57 mm Hg; from 87 to 144 mm Hg, resp.) and venous pressure (41 mm Hg, from 26 mm Hg to 67 mm Hg, resp.).

Conclusions Capillary pressure in the hallux increases upon dependency, however not as much as the toe blood pressure does. This study shows that activation of the peripheral vasoconstriction mechanisms appears to reduce the transmission of the rise in arteriolar pressure to the capillaries upon dependency.
Chapter 8 - Postural changes in capillary pressure

Introduction

Capillary microcirculation is essential for tissue nutrition, based on transcapillary exchange of fluids and solutes. The fluid exchange across the capillary membrane of a single, short section of a capillary, as initially proposed by Starling,\(^1\) is dependent on the transmural pressure gradient, in which the capillary blood pressure (CP) is a crucial factor.\(^2\) Microcirculatory perfusion in the skin of fingers and toes is mainly regulated by local mechanisms\(^3\), including the myogenic response of the precapillary arterioles after a change in transmural pressure,\(^4\) and the venuloarteriolar response (VAR), based upon a local axon reflex leading to arteriolar constriction when venular pressure is elevated.\(^5,6\) Disturbed constriction responses, leading to capillary hypertension, may cause edema formation.\(^3,7\)

Thus far the regulation of CP has been investigated in healthy and diseased subjects in the capillaries of the finger nail fold only, using a direct cannulation and a servo-nulling micropressure system.\(^8\) This direct method appeared to be a reliable technique to give valuable information about the (patho-)physiology of microcirculatory perfusion.\(^8,10\) Shore et al.\(^11\) demonstrated that elevation of systemic blood pressure through exercise did not change capillary pressure in the hand, and suggested that protective mechanisms minimize the transmission of changes in systemic blood pressure to the capillary bed, whereas an increase in venous pressure in the arm gave a pronounced rise in capillary pressure.\(^12\)

The regulation of capillary pressure in the foot is likely to be much more robust than in the hand in order to counteract the considerable changes in arterial and venous pressure upon a change in posture. On the other hand, various diseases (arterial and venous insufficiency, diabetic microangiopathy, etc.) mainly affect, or are more pronounced in, the microcirculation of the foot.\(^13-15\) To date, however, the regulatory mechanisms of capillary pressure in the foot have only been investigated by and in Levick and Michel using a direct, static capillary pressure measurement technique.\(^16\) They showed that capillary pressure can rise up to 100 mm Hg in standing position, and that in the dependent extremity there is a tight control of capillary pressure probably by arteriolar vasoconstriction. Furthermore, they showed that capillary pressure approaches venous pressure in the dependent position.\(^16\) However, this work was performed only in two volunteers with a static measurement system. Furthermore, dynamic measurements allow for additional analysis of the capillary pulse pressure and capillary pressure waveform during the cardiac cycle and yields information about pre-capillary resistance.\(^12,17\)

Knowledge about the regulation of the pre-capillary resistance in the foot may increase our understanding of the edema formation in patients suspected of capillary hypertension, e.g. venous insufficiency and diabetes mellitus, and the reappearance of postural vasoconstriction in the foot, e.g. after revascularization procedures that increase peripheral blood pressure. However, the (patho-) physiological knowledge of capillary pressure regulation in health and disease is mainly derived from investigations performed in the fingers.\(^11,17-19\) Therefore, in this study we investigated the effect of postural changes on capillary pressure and perfusion and in the hallux in healthy subjects by means of capillary microscopy, laser Doppler fluxmetry, and a servo-nulling micropressure system.
Subjects and methods

The left foot of twenty healthy volunteers was investigated after a 30 min acclimatization period in a temperature controlled environment (25 ± 1°C). All measurements were performed in the sitting and the supine position with the foot at heart level in random order. The height from toe to heart (represented by the fourth intercostal space) was measured in sitting position. Volunteers refrained from smoking and caffeine containing drinks for at least four hours before the measurement to avoid a possible effect on vascular tone. The investigation protocol was approved by the local medical ethical committee and conforms with the principles outlined in the declaration of Helsinki. Written informed consent was obtained from all volunteers.

Dynamic intracapillary blood pressure measurements were performed in the eponychium of the hallux of the left foot in the sitting and supine positions. Laser Doppler fluxmetry (PF 407, Periflux 4001, Perimed, Sweden; filter time 0.03 s) of the adjacent area, continuous blood pressure of the second toe (Finapres BP Monitor 2300, Ohmeda, Louisville, Co, USA) and ECG were assessed simultaneously, to validate the capillary pressure (figure 1 and 2). The laser Doppler was used to investigate total cutaneous perfusion, whereas the capillary red blood cell velocity was assessed to evaluate nutritive perfusion. Skin temperature of the toe (Monitor 78342A, Hewlett Packard, USA) was monitored to evaluate a possible variability due to temperature changes. All synchronous measurements (ECG, laser Doppler, temperature, capillary and continuous toe pressure) were sampled on-line and analyzed off-line by means of a data acquisition and analysis system (AcqKnowledge III and MP 100WSW, Biopac System, Inc., Santa Barbara, CA, USA). After this experiment the brachial (Dinamap Plus; Criticon, Tampa, Fl, USA) and ankle pressures (highest of dorsal

Figure 1. Photograph of measurement setup in sitting position
pedal artery and posterior tibial arteries at the level of the ankle) were measured with an 8 Mhz Doppler probe (PV lab, Stöpler, Electric Diagnostic Instruments, Burbank, CA) and a cuff (12 cm width) just above the ankle. The toe pressure was measured on the hallux of the same foot as the capillary pressure by means of photo-plethysmography (PPG; PV lab, Stöpler, Electric Diagnostic Instruments, Burbank, CA) and a digital cuff with a width depending on the diameter of the toe. A cuff width closest to 120% of the diameter of the hallux was chosen (cuff: 1.5, 2.5 or 3.3 cm, Hokanson, Bellevue, WA, USA). Additionally, peripheral venous blood pressure was measured in the great saphenous vein at the foot, by means of a 22 gauge venflon connected to a pressure transducer (Monitor 78342A, Hewlett Packard, USA) in 8 subjects in the same positions in which the capillary pressure measurements were performed.

**Intravital capillary microscopy**

The capillaries of the hallux nail fold were visualized by means of a capillary microscope with motor focusing in combination with a video circuitry as described before. Capillaries were punctured while visualized using a 10x objective (PL Fluotar, 10/0.30 Leitz Wetzlar Germany), and a digital camera (TM-6CN Pulnix America Inc., Sunnyvale, Ca, USA), giving a total magnification on a monitor (PM 931, Ikegami, Korea) of about 310x (screen: 180 x 136 mm = 0.58 x 0.44 mm skin area). The images were stored on videotape for off-line analysis of the capillary diameter (in μm), capillary density (in mm\(^{-2}\)), and capillary red blood cell velocity (RBCV; in μm/s) during the capillary pressure measurement as described before (Cap-Image software, Zeintl, Biomedical Engineering, Heidelberg, Germany).

**Figure 2.** Detail of the measurement setup: (1) micropipette, (2) laser Doppler, (3) skin thermometer, (4) manchet of continuous toe blood pressure measurement, (5) reference electrode, (6) lens of capillary microscope.
Capillary pressure was measured by a direct servo-nulling method. The principles of the measurement technique, circuit description, and calibration have been evaluated and described in detail before for the fingers, but are essentially the same for the measurements in the toes. In short, the capillary loops were punctured in the apex with micropipettes (tip diameter varying between 3 and 4 μm), filled with a 2M NaCl solution (with 10E/ml heparin to prevent plugging), connected to the servo-nulling micro pressure system (900A World Precision Instruments, Sarasota, FL, USA). The apparatus contains an electrical and an air circuit, which regulates the pressure inside the pipette so that it equals the pressure outside the tip. The electrical circuit is formed by a Wein bridge oscillator, which generates a 1000 Hz (sinusoidal voltage) constant carrier current through the microelectrode. An influx of blood into the capillary would change the impedance of the pipette. A pressure control driver will automatically adjust the microelectrode tip impedance to a change in pressure outside the tip of the pipette.

The cuticle and upper layer of the stratum corneum of the epidermis were removed to facilitate puncturing of the capillaries. The position of the tip of the pipette was adjusted so that flow through the capillary was visually unobstructed and a synchronous waveform was received (figure 3). A measurement was regarded valid when the capillary pulse pressure waves were in phase with the waveforms of the ECG, toe pressure and laser Doppler, whilst capillary flow was unobstructed for at least 5 s. The mean systolic, diastolic and mean pressures were derived from the valid interval. Furthermore, the capillary pulse pressure amplitude (CPPA), which can be used as a measure of the precapillary resistance, was defined as the difference between the diastolic and systolic peak. After the investigation any remaining shards were removed by wiping with a paper tissue and the puncture area was disinfected.

Figure 3. Example of a recording of the laser Doppler flux, capillary and second toe pressure (Finapres) and ECG in sitting (left panel) and supine (right panel) position. Note the difference in (especially capillary pressure) wave forms between both positions.
Statistics
The results are expressed in means with standard deviations after testing for skewness. Statistical analysis of possible differences between the sitting and supine positions in all measurements was evaluated using the paired t-test.

Results
In 13 out of 20 (65%) volunteers (mean age 34 ± 10 yrs.; 4 men and 9 women) acceptable measurements could be obtained in both positions. The number of failures diminished with growing experience and were caused by movement artefacts (n= 5) and thickness of the skin (n= 2). The duration of a complete investigation in both positions varied between 2 to 4 hours. If the flow through the capillary was visually unobstructed, the pulse contour of the pressure could be visualized clearly, showing a steep upstroke in phase with the other recordings (figure 3). The difference in pulse contour between the sitting and supine positions due to a change in pre-and post-capillary resistance is clearly visible in these figures. The duration of one continuous pressure recording varied from 5 s. to 6 min, and was typically stable over time (figure 4).

Figure 4. Typical example of the variation over a longer recording period of ECG, laser Doppler, capillary and second toe pressure (Finapres).
Capillary pressure in the sitting position was significantly higher than in the supine position (see Table 1 and figure 5). The same was true for the toe, ankle, and venous pressures, whereas brachial pressures and skin temperature did not change significantly during the investigation. However, the rise in systolic capillary pressure (mean 32 mm Hg) was significantly (p=0.001) less than the rise in systolic ankle and toe pressures (both mean 57 and 57 mm Hg, see Table 1), but less than the rise in venous pressure (mean 41 mm Hg). There was a significant difference between the arterial-capillary blood pressure fall between supine and sitting position (56 and 81 mm Hg, respectively). Capillary systolic pressure rose to up to 100 mm Hg in the sitting position. However, the remaining microcirculatory parameters (CPPA, capillary density, RBCV, capillary diameter, LDF) did not change significantly upon the change in posture, indicating that capillary volume flow and total skin flow did not alter substantially by change in posture (Table 1).

**Discussion**

This study shows that dynamic capillary pressure measurements are feasible in the capillary nail fold of the toe, both in the sitting and supine positions. Even the pulse contour of the blood pressure is clearly visible with this method. Secondly, this study confirms the observations of Levick and Michel\(^\text{16}\) that the capillary pressure increases substantially in sitting position, but not as much as the increase in arterial pressure. This is probably due to activation of pre-capillary (arteriolar) vasoconstriction mechanisms.

**Table 1. Measurement parameters in the sitting and supine positions**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Supine (sd)</th>
<th>Sitting (sd)</th>
<th>Sitting-supine difference (sd)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brachial systolic (mm Hg)</td>
<td>117 (7)</td>
<td>118 (8)</td>
<td>2 (10)</td>
<td>0.58</td>
</tr>
<tr>
<td>Brachial diastolic (mm Hg)</td>
<td>70 (7)</td>
<td>73 (4)</td>
<td>3 (7)</td>
<td>0.12</td>
</tr>
<tr>
<td>Ankle systolic (mm Hg)</td>
<td>118 (7)</td>
<td>175 (8)</td>
<td>57 (9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Toe systolic (mm Hg)</td>
<td>87 (17)</td>
<td>144 (18)</td>
<td>57 (17)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Toe systolic Finapres (mm Hg)</td>
<td>98 (16)</td>
<td>171 (25)</td>
<td>73 (28)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Toe mean Finapres (mm Hg)</td>
<td>65 (15)</td>
<td>126 (18)</td>
<td>61 (16)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Toe diastolic Finapres (mm Hg)</td>
<td>50 (16)</td>
<td>106 (17)</td>
<td>56 (14)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Capillary systolic (mm Hg)</td>
<td>39 (12)</td>
<td>71 (11)</td>
<td>32 (10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Capillary mean (mm Hg)</td>
<td>32 (8)</td>
<td>63 (8)</td>
<td>32 (7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Capillary diastolic (mm Hg)</td>
<td>28 (8)</td>
<td>59 (8)</td>
<td>31 (6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pressure fall (mm Hg)</td>
<td>56 (17)</td>
<td>81 (20)</td>
<td>25 (19)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Capillary density (/mm(^2))</td>
<td>55 (12)</td>
<td>58 (14)</td>
<td>3 (15)</td>
<td>0.56</td>
</tr>
<tr>
<td>CPPA (mm Hg)</td>
<td>11 (7)</td>
<td>12 (5)</td>
<td>0.1 (5.1)</td>
<td>0.50</td>
</tr>
<tr>
<td>RBCV (mm/s)</td>
<td>0.19 (0.04)</td>
<td>0.19 (0.07)</td>
<td>0.0 (0.06)</td>
<td>1.0</td>
</tr>
<tr>
<td>Capillary diameter ((\mu m))</td>
<td>9.5 (1.7)</td>
<td>9.1 (1.0)</td>
<td>-0.4 (1.8)</td>
<td>0.51</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>26 (2)</td>
<td>26 (2)</td>
<td>0.5 (1.3)</td>
<td>0.26</td>
</tr>
<tr>
<td>LDF (Volts)</td>
<td>0.18 (0.17)</td>
<td>0.22 (0.21)</td>
<td>0.05 (0.25)</td>
<td>0.50</td>
</tr>
<tr>
<td>High heart-toe (cm)</td>
<td>0</td>
<td>79 (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous pressure (mm Hg)</td>
<td>26 (3)</td>
<td>67 (5)</td>
<td>41 (4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High heart-toe (cm)</td>
<td>0</td>
<td>86 (6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed in means with standard deviations, and differences are tested with the paired t test. Abbreviations: Pressure fall: pressure difference between toe pressure and mean capillary pressure; CPPA: capillary pulse pressure amplitude, RBCV: capillary red blood cell velocity; LDF: laser Doppler fluxmetry.
In this study systolic capillary pressure in the toe while sitting was found to be rather high (70 up to 100 mm Hg), which is much higher than commonly known. Our results correspond with the static capillary pressure measurements using a manometric technique as performed by Levick and Michel, who found a capillary pressure of 67 mm Hg when the hydrostatic pressure difference between heart and capillary was 80 mm Hg, and pressures up to 100 mm Hg in the standing position. This increased pressure in the sitting position considerably influences the forces governing fluid movement across the capillary wall, since the capillary pressure is a crucial factor in this process, and is bound to lead to interstitial edema formation. The fluid exchange across the capillary membrane of a single, short section of a capillary, as initially proposed by Starling, is dependent of the transmural pressure gradient. An increase in hydrostatic pressure causes extravascular fluid accumulation and edema formation. This process is linearly related to the ortho- or hydrostatic pressure. An increase in mean capillary pressure from 32 to 64 mm theoretically increases the fluid passage across the capillary wall (fluid filtration rate rises from 0.1 to about 0.4 μm²/mm²). Under physiological situations the orthostatic venous pressure is reduced by the calf muscle pump. This in turn might lower the capillary pressure, and seems to be an essential factor in the prevention of edema formation, provided the venous valves are sufficient. Unfortunately, the effect of the calf muscle pump could not be evaluated in our set-up, because even an isometric contraction of the calf muscle may cause movement artifacts that break the tip of the pipette.

The height difference between heart and toe was 79 cm, causing an orthostatic pressure equal to 58 mm Hg, which corresponds with the increase in ankle and toe blood pressures (59 mm Hg). Ubbink et al. studied the effect of posture on skin
capillary perfusion in the foot and concluded that post-capillary pressure appears to be an important factor in the regulation of capillary perfusion, because an increase in venous resistance mimics the effects of leg dependency.\textsuperscript{23} Previous investigations showed that microcirculatory perfusion in the foot changes upon a change in posture, which results in a reduced red blood cell velocity\textsuperscript{23,27}, resulting in a decreased laser Doppler flux (LDF), probably caused by arteriolar vasoconstriction.\textsuperscript{28,29} However, these observations are not confirmed in the present study. The LDF did not showed any significant difference because LDF was measured at the dorsum of the toe which is known to have a smaller effect on postural changes than the pulp of the toe, since the pulp has a large number of arteriovenous anastomoses, which are rare at the dorsal site.\textsuperscript{3,30} Surprisingly we did not observed a significant reduction of RBCV in sitting position, which might be caused small group of patients, the measurement time (during the pressure measurement), or the used measurement technique.

The present study showed direct evidence for arteriolar vasoconstriction. The pressure fall between artery and capillary increased significantly, whereas flow (RBCV and capillary diameter, and laser Doppler) remained unchanged (table 1). This suggests that activation of the postural (arteriolar) vasoconstriction response apparently reduces the transmission of the digital arterial pressure to the capillaries.\textsuperscript{5,6,31} This is confirmed by an increase in the pre to post capillary resistance ratio \( \{(P_a-P_c)/(P_c-P_v)\} \) reflecting the distribution of resistance in the local circulation.\textsuperscript{16} We calculated this ratio from the systolic arterial toe, capillary, and venous foot pressures. The ratio in the sitting position (ratio: 18) is much higher than in the supine position (ratio: 4), which means an increase in pre-capillary (arteriolar) resistance in the sitting position. This would be in agreement with our hypothesis that arteriolar vasoconstriction mechanisms (probably mainly the venoarteriolar reflex) prevent transmission of the increase in arterial pressure to the capillaries.\textsuperscript{5,6} Although this could not be confirmed by a reduction in CPPA, a reduction in the number of perfused capillaries, or a reduction in laser Doppler skin perfusion. Therefore the present observations indicate that flow regulation outweighs pressure regulation in the microcirculation.

In summary, this is the first study measuring dynamically the capillary pressure in the toe. Our results indicate that the systolic capillary blood pressure can rise substantially (up to 100 mm Hg) upon leg dependency, but remains lower than the increase in toe systolic pressure. Apparently, activation of the peripheral vasoconstriction responses reduces the transmission of the arteriolar pressure to the capillaries, and probably contributes as an edema preventing factor. This technique seems suitable to provide valuable information about the pathophysiology of microvascular disorders in the foot.

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Chapter 8 - Postural changes in capillary pressure

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