Withstanding the flow

*Human cardiovascular control during postural challenges*

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Chapter 5

ORTHOSTATIC LEG BLOOD VOLUME CHANGES ASSESSED BY NEAR-INFRARED SPECTROSCOPY


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Chapter 5

ABSTRACT

Background

Standing up shifts blood to dependent parts of the body and blood vessels in the leg become filled. The orthostatic blood volume accumulation in the small vessels is relatively unknown, whereas these may contribute significantly. We hypothesized that in healthy humans exposed to the upright posture volume accumulation in small blood vessels significantly contributes to the total fluid volume accumulated in the legs.

Methods

Considering that near-infrared spectroscopy (NIRS) tracks postural blood volume changes within the small blood vessels of the lower leg we evaluated the NIRS determined changes in oxygenated (Δ[oxy-Hb]), deoxygenated (Δ[deoxy-Hb]) and total hemoglobin (Δ[total Hb]) tissue concentration and in total leg volume by strain gauge plethysmography during 70° head-up tilt (HUT) (n=7). In a second experiment spatial and temporal reproducibility were evaluated with three NIRS probes applied on two separate days (n=8).

Results

In response to HUT an initially fast increase in [oxy-Hb] was followed by a gradual decline, while [deoxy-Hb] increased continuously. The increase in [total Hb] during HUT was closely related to the increase in total leg volume (r²=0.95±0.03). After tilt back [oxy-Hb] declined below and [deoxy-Hb] remained above baseline, whereas all NIRS signals gradually returned to baseline. Spatial heterogeneity was observed and in two probes [total Hb] highly related between days (r²=0.92±0.09 and 0.91±0.12), but less in the third probe (r²=0.44±0.36).

Conclusion

The results suggest a non-linear accumulation of blood volume in the small vessels of the leg with an initial fast phase followed by a more gradual increase at least partially contributing to the relocation of fluid during orthostatic stress.
The immediate circulatory event upon assumption of the upright position, either passive or active, is a gravitational displacement of blood to dependent regions of the body and a fall in venous return.\textsuperscript{18, 19} \(300-800\) ml of blood is shifted from the chest to lower parts of the body during orthostatic stress\textsuperscript{20, 21} and \(\sim50\%\) of that shift takes place within the first few seconds.\textsuperscript{230, 231} Passive head-up tilt (HUT) decreases activity of technetium labelled erythrocytes (\(99mTc\)) over the thorax by \(\sim25\%\), whereas the \(99mTc\) activity over the thigh increases.\textsuperscript{19} This intravascular part of the orthostatic shift in volume is supposed to reflect a rapid filling of capacitance vessels in the lower body,\textsuperscript{22, 23} while the further fall in circulating blood volume during sustained HUT is considered to be driven by enhanced capillary transmural pressure with fluid filtration into the interstitial space.\textsuperscript{19, 24, 25}

Prolonged accumulation of blood in dependent vessels has, however, been described.\textsuperscript{401, 402} Traditionally, strain gauge plethysmography is used to evaluate orthostatic fluid shifts from the upper body to the lower limbs\textsuperscript{6}. Intra- vs. extravascular volume changes cannot be differentiated by this technique and generally, assumptions on the time course of volume changes in these separate compartments have to be made. Furthermore, at high pressures only about half of the orthostatic increase in leg volume is explained by blood volume in the large veins, whereas the small blood vessels may contribute significantly.\textsuperscript{403, 404}

We hypothesized that in healthy humans exposed to the upright posture volume accumulation in small blood vessels significantly contributes to the total fluid volume accumulated in the legs. We considered that near-infrared spectroscopy (NIRS) can be used to track intravascular orthostatic volume changes within the small blood vessels of the lower leg\textsuperscript{57, 405, 406}. Therefore we evaluated the NIRS determined changes in oxygenated (\(\Delta[\text{oxy-Hb}]\)), deoxygenated (\(\Delta[\text{deoxy-Hb}]\)) and total hemoglobin tissue concentration (\(\Delta[\text{total Hb}]\)) and in total leg volume by plethysmography during the passive orthostatic blood volume accumulation in the legs of healthy subjects.

**METHODS**

Healthy subjects were included after oral and written informed consent was obtained as approved by the Medical Ethical Committee of the Academic Medical Center of the University of Amsterdam. Subjects abstained from alcohol and caffeine from 24 h before the study until completion.

**Measurements**

Measurements were performed on a tilt table and subjects were supported by a footboard. NIRS was applied to track changes in leg blood volume by orthostatic stress.\textsuperscript{405, 407, 408} The change in [total Hb], as the sum of oxygenated ([oxy-Hb]) and de-oxygenated ([deoxy-Hb]) hemoglobin tissue concentration, was taken to represent deviations of calf blood volume. A two-wavelength (780 and 850 nm) multi-channel continuous wave NIRS system (Oxymon, Artinis Medical Systems BV, The Netherlands) sampling at 10 Hz was used. The NIRS optodes were fixed with an inter-optode distance of 4.5 cm.

Hemodynamic monitoring included recording of blood pressure by a servo-controlled photo-plethysmograph (Portapres\textsuperscript{®}, FMS, Amsterdam, the Netherlands) with the cuff placed on the middle phalanx of the left middle finger that was kept at heart level and the signal was A/D
converted at 100 Hz for off-line analysis. Beat-to-beat values for mean arterial pressure (MAP) were derived as the integral over one heart beat and heart rate (HR) was the inverse of the pulse interval. Beat-to-beat stroke volume (SV) was estimated from the arterial pressure wave according to the Modelflow method: integration of the aortic flow waveform per beat provides left ventricular SV. Cardiac output (CO) is SV times HR, and total peripheral resistance (TPR) is MAP divided by CO (BeatScope 1.1, FMS, Amsterdam, the Netherlands).

Near-infra red spectroscopy and plethysmography

Eight subjects were studied using simultaneous recordings of NIRS and strain gauge plethysmography (Medasonics SPG-16) of the right lower leg. Signals were sampled at 10 Hz and stored on hard disk. The NIRS probe covered the posterolateral part of the soleus muscle at the level of maximal calf circumference with the transmitting and receiving optodes aligned with the vertical axis of the leg. Between the NIRS optodes a mercury in strain gauge was applied around the lower leg.

Following instrumentation and a 5 min baseline measurements at supine rest subjects were tilted 70° head-up (t = 0). Subjects were tilted back after 10 min and measurements continued for another 10 min in the supine position.

Near-infra red spectroscopy reproducibility

Eleven subjects were studied at the same time of the day (±15 min) on two separate days. The location of the NIRS optodes was marked for placement on the second day of measurement. An LBNP box was tightened over the iliac crest with the subjects supine on a manually operated tilt table. In order to evaluate spatial differences three NIRS probes were positioned along the lateral surface of the right lower leg (Figure 5.1): probe 1 was placed over the anterolateral muscle compartment, including the peroneus longus, extensor digitorum longus and tibialis anterior muscles, while the two other probes were located at the upper (probe 2) and lower (probe 3) parts of the soleus muscle.
Following instrumentation and a 5 min baseline measurements at supine rest subjects were tilted 70° head-up (t = 0) and after 20 min, the orthostatic stress was intensified by adding 20 mmHg LBNP for 10 min. Subjects were tilted back and LBNP was released immediately if presyncopal symptoms revealed.

The orthostatic increase in lower leg [total Hb] was expressed by a double exponential model describing the initial rapid vascular distention followed a prolonged increase in blood volume that can be described by a first order transfer function (Eq. 1).\textsuperscript{235}

\[
\Delta[\text{total Hb}](t) = a_1 \left(1 - e^{-\frac{t}{\tau_1}}\right) + a_2 \left(1 - e^{-\frac{t}{\tau_2}}\right) \quad (1)
\]

where \(a_1\) and \(a_2\) are amplitudes and \(\tau_1\) and \(\tau_2\) time constants of the two phases of orthostatic blood volume accumulation in the lower leg, whereas \(t\) is time in seconds from onset of HUT.
**Statistical analysis**

The relation between $\Delta[\text{total Hb}]$ by NIRS and total leg volume by strain gauge plethysmography during HUT was tested by Pearson’s correlation coefficient. Within-subject reproducibility over the two days of investigation was evaluated by linear regression of the time courses of $\Delta[\text{total Hb}]_{\text{day1}}$ vs. $\Delta[\text{total Hb}]_{\text{day2}}$ for each subject. Two-way ANOVA for repeated measures was used to identify differences in $\Delta[\text{total Hb}]$ between probes and days. Paired t-test or the Wilcoxon Signed Rank test were used to analyze changes during the experiments. Data are presented as mean±SD and the level of statistical significance was set at $p<0.05$.

**RESULTS**

*Near-infra red spectroscopy and plethysmography*

Data from 1 subject suffering from early pre-syncope were excluded, and data from 7 subjects (4 women; age: 27 ± 3 years; height: 172 ± 7 cm; weight: 64 ± 4 kg; body mass index 22 ± 1 kg/m²) became available for analysis. Signals from NIRS and strain gauge plethysmography were stable in the supine position (Figure 5.2).

In response to HUT, $[\text{total Hb}]$ and total leg volume increased with an initial rapid phase followed by a more gradual increase. The increases in $[\text{total Hb}]$ and total leg volume related closely ($r^2 = 0.95 \pm 0.03$). The HUT induced $\Delta[\text{oxy-Hb}]$ comprised a initial rapid increase followed by a gradual decline. $\Delta[\text{deoxy-Hb}]$ demonstrated a consistent gradual increase after HUT. After tilt back both $[\text{total Hb}]$ and total leg volume rapidly decreased with $[\text{total Hb}]$ reaching baseline and total leg volume remaining above baseline. $[\text{oxy-Hb}]$ declined below and $[\text{deoxy-Hb}]$ remained above baseline, whereas all NIRS signals gradually returned to baseline within 10 min.
Near-infra red spectroscopy reproducibility

Data from 1 subject suffering from early pre-syncope and from 2 subjects with movement artifacts in the NIRS signal were excluded from further analysis. Consequently, data were available for 8 subjects (6 women; age: 29 ± 8 years; height: 174 ± 12 cm; weight: 69 ± 12 kg; body mass index 23 ± 2 kg/m²). The increase in HR, MAP and TPR and the decline in SV in response to HUT were comparable between days (Table 5.1; Figure 5.3).
Table 5.1  Hemodynamic variables on day 1 four minutes before and eight minutes after head-up tilt

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rest -4 min</th>
<th>HUT +8 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>71 ± 12</td>
<td>83 ± 14*</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>67 ± 8</td>
<td>81 ± 9*</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>81 ± 18</td>
<td>64 ± 14*</td>
</tr>
<tr>
<td>CO (l·min⁻¹)</td>
<td>5.3 ± 0.7</td>
<td>5.1 ± 0.9</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>48 ± 12</td>
<td>45 ± 13</td>
</tr>
<tr>
<td>TPR (mmHg·ml⁻¹·s)</td>
<td>1077 ± 146</td>
<td>1330 ± 226*</td>
</tr>
</tbody>
</table>

HUT: head-up tilt; MAP: mean arterial pressure; HR: heart rate; SV: stroke volume; CO: cardiac output; PP: pulse pressure; TPR: total peripheral resistance. *p<0.001 vs. rest -4 min. Values are means ± SD

Table 5.2  Within-subject reproducibility of changes in total hemoglobin tissue concentrations between day 1 and day 2.

\[
\Delta[\text{total Hb}]_{\text{day 2}} = y_0 + a \cdot \Delta[\text{total Hb}]_{\text{day 1}}
\]

<table>
<thead>
<tr>
<th>Probe</th>
<th>(y_0) ± SD</th>
<th>(a) ± SD</th>
<th>(r^2) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probe 1</td>
<td>-0.7 ± 2.4</td>
<td>1.70 ± 3.32</td>
<td>0.44 ± 0.36</td>
</tr>
<tr>
<td>Probe 2</td>
<td>1.0 ± 3.4</td>
<td>1.04 ± 0.26</td>
<td>0.92 ± 0.09</td>
</tr>
<tr>
<td>Probe 3</td>
<td>-0.2 ± 3.1</td>
<td>0.97 ± 0.25</td>
<td>0.91 ± 0.12</td>
</tr>
</tbody>
</table>

\([\text{total Hb}]_{\text{day 2}}\): total hemoglobin tissue concentration on day 2; \([\text{total Hb}]_{\text{day 1}}\): total hemoglobin tissue concentration on day 1; \(y_0\): y-intercept of the regression model; \(a\): slope of the regression model; \(r^2\): coefficient of determination. Probe 1 placed over the anterolateral muscle compartment, while probes 2 and 3 represent the soleus muscle. Values are means ± SD
After HUT the increase in $\Delta[\text{total Hb}]$ closely related between the two days in probes 2 and 3 ($r^2 = 0.92 \pm 0.09$ and $0.91 \pm 0.12$, respectively), but less in probe 1 ($r^2 = 0.44 \pm 0.36$; Table 5.2). In response to HUT, [total Hb] followed the hypothesized double exponential model at all three probe locations (Table 5.3; Figure 5.4). Following the initial fast phase, [total Hb] gradually increased further for 6 (probe 1: $\tau_2 = 80 (68 – 138)$ s) to 15 min (probe 2: $\tau_2 = 175 (123 – 212)$ s). No interaction effect between days and probes was observed.

**Figure 5.3** Mean arterial pressure, heart rate, stroke volume and total hemoglobin tissue concentration responses to head-up tilt on two separate days. MAP: mean arterial pressure; HR: heart rate; SV: stroke volume; $\Delta[\text{total Hb}]$: change in total hemoglobin tissue concentration; HUT: head-up tilt. $\Delta[\text{total Hb}]$ from probe 3, day 1: solid lines, day 2: dotted lines, grey areas are SEM.
Table 5.3 Modeled changes in total hemoglobin tissue concentration (Δ[total Hb]) in the calf after head-up tilt.

\[ \Delta[\text{total Hb}](t) = a_1 \left( 1 - e^{-\frac{t}{\tau_1}} \right) + a_2 \left( 1 - e^{-\frac{t}{\tau_2}} \right) \]

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Probe 1</td>
<td>0.95 (0.81 – 0.97)</td>
</tr>
<tr>
<td>( r^2 )</td>
<td>Probe 2</td>
<td>0.98 (0.97 – 0.99)</td>
</tr>
<tr>
<td></td>
<td>Probe 3</td>
<td>0.99 (0.98 – 0.99)</td>
</tr>
<tr>
<td></td>
<td>Probe 1</td>
<td>1.9 (0.6 – 3.8)</td>
</tr>
<tr>
<td>( \sigma_1 )</td>
<td>Probe 2</td>
<td>7.6 (0.7 – 8.0)</td>
</tr>
<tr>
<td></td>
<td>Probe 3</td>
<td>4.2 (3.1 – 8.5)</td>
</tr>
<tr>
<td></td>
<td>Probe 1</td>
<td>3.1 (2.1 – 3.8)</td>
</tr>
<tr>
<td>( \tau_1 )</td>
<td>Probe 2</td>
<td>3.6 (2.9 – 7.8)</td>
</tr>
<tr>
<td></td>
<td>Probe 3</td>
<td>3.1 (2.6 – 5.7)</td>
</tr>
<tr>
<td></td>
<td>Probe 1</td>
<td>2.9 (1.3 – 7.4)</td>
</tr>
<tr>
<td>( \sigma_2 )</td>
<td>Probe 2</td>
<td>20 (18 – 24)</td>
</tr>
<tr>
<td></td>
<td>Probe 3</td>
<td>15 (13 – 18)</td>
</tr>
<tr>
<td></td>
<td>Probe 1</td>
<td>80 (68 – 138)</td>
</tr>
<tr>
<td>( \tau_2 )</td>
<td>Probe 2</td>
<td>175 (123 – 212)</td>
</tr>
<tr>
<td></td>
<td>Probe 3</td>
<td>174 (120 – 271)</td>
</tr>
</tbody>
</table>

\( a \): asymptote; \( \tau \): time constant; \( t \): time in seconds from head-up tilt. Data are medians (IQR). [total Hb]: total hemoglobin tissue concentration. Probe 1 placed over the anterolateral muscle compartment, while probes 2 and 3 represent the soleus muscle.
The increase in [total Hb] 1 min after HUT was larger at probes 2 and 3 compared to probe 1 and this difference remained after 8 min (Figure 5.5). At 8 min of HUT, Δ[total Hb] differed between all three probes. Furthermore, location specific time courses of Δ[oxy-Hb] and Δ[deoxy-Hb] following HUT were observed (Figure 5.6).
Data from two subjects were excluded from the analysis of responses to HUT+LBNP because of early tilt back to the supine position due to pre-syncopal complaints and insufficient quality of the NIRS signals. In 6 subjects addition of LBNP to HUT increased [total Hb] and reduced SV further (Figure 5.7).

**Figure 5.6** Orthostatic changes in lower limb oxygenated, de-oxygenated and total hemoglobin tissue concentrations. HUT: head-up tilt; \([O_2Hb]\): oxygenated hemoglobin tissue concentration; \([HHb]\): de-oxygenated hemoglobin tissue concentration; \([Hb]\): total hemoglobin tissue concentration. Solid lines are means on day 1, dotted lines represent means on day 2. Grey area’s indicate one-directional SEM’s.
DISCUSSION

This study addressed the time course of the orthostatic accumulation of lower leg blood volume in small blood vessels using NIRS. We identified a rapid increase in total hemoglobin tissue concentration followed by a gradual increase, which together fitted a biphasic exponential model representing a prolonged filling of the small blood vessels in the leg. The present data suggest that the continuing increase in total leg volume in response to

**Figure 5.7** Hemodynamic and total hemoglobin tissue concentration responses to addition of lower body negative pressure to head-up tilt (n=6). HUT: head-up tilt; LBNP: lower body negative pressure (set at 20 mmHg after 20 min HUT); MAP: mean arterial pressure; HR: heart rate; SV: stroke volume; Δ[total Hb]: change in total hemoglobin tissue concentration at Probe 3. Black lines: means; grey areas: SEM.
orthostatic stress is explained, at least in part, by blood volume increase in the small blood vessels.

An initial fast phase of volume redistribution from the thorax to the legs has been reported to be followed by a slow phase involving fluid filtration into the interstitial space. The present data, however, suggest that this slow phase encompasses a gradual increase in intravascular volume in addition to the traditionally proposed movement of plasma into the interstitial space as described by plethysmography incorporating both intra- and extravascular fluid changes. Only part of the increase in leg volume is explained by the orthostatic increase in blood volume in the large veins. The data of this study, as previously proposed by Cirovic et al., indicate that the increase in leg volume results from an increase in blood volume in smaller blood vessels as well. Near-infrared photons penetrate biological tissues and are absorbed mainly by hemoglobin in the capillary, arteriolar and venular beds, and by myoglobin. The concentration of total Hb is determined from the amount of near-infrared light absorbed in the transilluminated tissue, of which the amount of myoglobin in the investigated muscles is considered not to respond to orthostatic stress. The ratio between chromophores and solvent in the tissue may decline by accumulating fluid (solvent) in the interstitial space as a result of enhanced transmural pressure with potentially some underestimation of the increase in [total Hb] (chromophore) by NIRS.

The time course of the postural shift in [total Hb] in the calf revealed a biphasic exponential increase with a time constant of almost three minutes, implying prolonged accumulation of erythrocytes. Similarly, an increase in blood volume for up to five minutes was suggested by accumulation of 133Xenon and an increase in 99mTc-labeled erythrocytes in the leg following venous occlusion in three subjects. Leg vascular resistance increases in response to HUT with a reduction in leg blood flow. Separation of Δ[total Hb] into its components (Δ[oxy-Hb] and Δ[deoxy-Hb]) provides a detailed evaluation of the HUT-induced leg blood volume increase. The postural reduction in calf [oxy-Hb] may represent reflex arterial vasoconstriction as a decrease in [oxy-Hb] correlates with leg blood flow and inversely with sympathetic activity. We speculate that the magnitude of this reduction in [oxy-Hb] may reflect the contribution of local vasoconstriction in the leg to the total increase in TPR and hence to blood pressure regulation in the upright posture. The early rapid increase in lower leg [total Hb] originated mainly from an increase in [oxy-Hb], whereas the following gradual increase originated from the increase in [deoxy-Hb]. This may indicate that the blood initially accumulates in the small arteries and arterioles of the leg followed by a gradual increase of blood volume in the veins and venules. Noteworthy, after tilt back [oxy-Hb] decreased below baseline with a subsequently slow recovery suggestive for a gradual fading of vasoconstriction. In contrast, [deoxy-Hb] gradually declined to baseline suggesting a prolonged increase in venous blood volume above baseline after HUT.

Cirovic et al. suggested that the elevated hydrostatic pressure induced by HUT opens smaller veins or venular channels explaining the extended increase in blood volume. We speculate that the prolonged orthostatic increment in leg [total Hb] is explained by venous stress-relaxation during maintained pressure elevation. This is supported by the gradual increase and subsequent decline of [deoxy-Hb] during HUT and tilt back, respectively. Vascular stress-relaxation is recognized in both experimental models and humans, but its influence on orthostatic blood redistribution to the lower body has not previously
been investigated. Venous stress relaxation during HUT was suggested as to explain the absence of the characteristic transient decline in systolic pressure that is observed during active standing only when leg muscle groups are activated. Accumulation of blood in the lower leg may be limited by the maximal vessel wall stretch. However, adding LBNP to HUT further increased lower limb $\Delta[\text{total Hb}]$ together with a continuing decrease in SV excluding vascular distensibility as limiting factor for accumulation of blood in the lower leg.

During HUT, the extent and reproducibility of the NIRS-determined increase in lower limb blood volume depended on the location of the probe, but they universally demonstrated an initial fast increase followed by a more gradual increase. Yet, further strain on the vessels by LBNP enhanced the accumulation of blood in the lower leg, demonstrating a balance between vascular tone and transmural pressure. Spatial heterogeneity in blood flow within and between muscles has been related to functional and anatomical factors such as muscle fiber type, capillary density, intramuscular pressure, and muscle architecture.

Reproducibility of orthostatic leg blood volume redistribution was location-dependent with high within-subject reproducibility for the probes covering the soleus muscle, but a lower reproducibility across the mm. peroneus longus, extensor digitorum longus and tibialis anterior muscles. Whereas the mechanism of this discrepancy is uncertain we consider variable engagement of antigravity muscles during footboard supported HUT as a biologically plausible explanation.

We suggest that activity by multiple muscles in the heterogeneous anterolateral compartment generates a more variable impact of the muscle pump. In addition, magnetic resonance imaging revealed that the reactive hyperemic response following arterial occlusion of the leg is most prominent in the highly vascularized tissue of the soleus muscle with a high percentage of type 1 muscle fibers. In contrast, the unyielding anterolateral fascial compartment confines a larger proportion of less vascularized type 2 muscle fibers. This potentially accounts for the smaller increase in [total Hb] reflecting limited blood accumulation within this compartment. The present study suggests that NIRS can be applied to monitor blood volume changes in the small blood vessels of the leg and emphasizes the importance of probe placement.

In summary, the results suggest a non-linear accumulation of blood volume in the small vessels of the leg with an initial fast phase followed by a more gradual increase at least partially contributing to the relocation of fluid during orthostatic stress.