Improving the clinical applicability of laser Doppler perfusion monitoring
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Comparison of laser Doppler perfusion monitors: a study on patients and healthy controls

Fernando Morales, Judith Blaauw, Andries J. Smit, Gerhard Rakhorst, Reindert Graaff

Summary

Two different commercial multi-channel laser Doppler perfusion monitors were compared in a clinical post-occlusive reactive hyperaemia test (PORH). Flux signals and response times from the PORH test were studied at two foot locations. The perfusion monitors assessed skin blood perfusion simultaneously at the dorsum of the foot and at the pulp of the hallux, using two multifibre probes. Measurements were performed on ten patients with peripheral arterial obstructive disease, ten patients with diabetes mellitus and ten controls. Resting and maximum fluxes were studied as well as three characteristic time parameters from the hyperaemic response. In both foot locations, different flux values were assessed at the various fibre distances. Time parameters at each foot location were practically the same for both instruments and all fibre distances. The time from reflux after occlusion release to resting flux, $t_{RF}$, differed significantly between controls and PAOD patients at the dorsum of the foot. No differences in flux values were found between patient and control groups. Of all investigated variables, $t_{RF}$ had the largest discriminating value between patients and controls, and was similar for both monitors. Flux results from different monitors or fibre distances need correction to allow comparison.
4.1 Introduction

Laser Doppler perfusion monitoring (LDPM) is a well-known technique to assess the tissue perfusion. The main advantage of this technique is the possibility of continuous monitoring of the rapid changes of blood flow through a small volume of tissue in a non-invasive way. In 1975, Stern proposed the clinical use of laser Doppler flowmetry [24] which led to the development of clinically oriented instruments using a He-Ne laser emitting light of 632.8 nm [3,6,12,23]. Nowadays, there are several manufacturers delivering LDPM monitors having in common the use of a diode laser that produces monochromatic light of 780 nm. This wavelength allows LDPM measurements to be almost unaffected by the oxygen saturation of blood [12,27]. This difference in wavelength should be taken into account when comparing results obtained with devices that used a He-Ne laser instead [2,20], because the oxygen saturation may influence those results.

LDPM monitors share a common basic functioning principle [6,24], but also have some differences [12]. An important difference between the devices is the methods for signal processing used by each manufacturer [12]. Another difference is the calibration liquid supplied by each manufacturer. Typically, the calibration liquid is an aqueous suspension of polystyrene spheres, but every manufacturer use different size and concentration of spheres. The Brownian movement of these particles is the origin of flux signal that is used as a reference. Alternative methods for calibration of LDPM monitors have been proposed [11,13]. These two differences lead to a somewhat dissimilar signal output of the monitors even if they measure simultaneously in the same medium [11,21].

Other limitations that need to be solved include lack of quantitative units [22], and the existence of the instrumental zero and biological zero [26]. Moreover, there is a need for standardised clinical protocols that would allow reproducible measurements.

In LDPM, the measuring depth is largely affected by the separation between the illuminating and detecting fibres [8,9,19]. This effect has been studied for different probes arrangements in vitro [8,11,14] and in vivo [4,5,7,10,16]. Although these studies have found that the LDPM flux signals vary with fibre separation, LDPM manufacturers offer a variety of probes with different fibre arrangements. Nevertheless, very little is still known of the influence of fibre separation in the clinical application of LDPM, or the possible advantages some fibre arrangements might have.

Currently, there are many LDPM monitors being used for research and, eventually, as a clinical tool. Therefore, it is important to know whether the
measurements obtained by the actual devices or with the previous genera-
tions are comparable. Several studies compared two or three LDPM de-
vices, but did so only under laboratory conditions [2,15] or in animal ex-
periments [20,22]. Ideally, an adequate performance comparison of moni-
tors is made by sharing only one probe with various monitors and re-
cording tissue perfusion simultaneously [21]. A comparison can be
achieved also by means of a multiple-fibre probe. However, it is very
important that every monitor assesses the perfusion signal using the same
fibre distance between the illuminating and detecting fibres, because that
distance has a major influence on the results [9,11,14], depending on the
methodology of calibration that is applied.

The present study aims to compare and evaluate the flux signals recorded
with two different LDPM multi-channel monitors in a clinical situation over
a range of conditions with different perfusion levels in patients with periph-
eral arterial obstructive disease, in patients with diabetes mellitus, and in
control subjects. By means of two LDPM probes with multiple detection fi-
bres, the influence of fibre distance is evaluated in these patient groups.
The simultaneously obtained flux signals from two foot locations are also
compared.

4.2 Methods

4.2.1 Equipment

Two LDPM monitors were used: a Pf4001 (dual-channel, Perimed AB, Jär-
fälla, Sweden) and a moorLAB system (quad-channel, Moor Instruments
Ltd., Axminster, UK). Thus, a total number of six channels were available,
each consisting of one laser and one detection unit. From these, five detec-
tion units and two lasers from the moorLAB were actually used for this
study. Both LDPM monitors were set to their minimum time constant (0.2 s
for Pf4001, 0.03 s for moorLAB). The maximal cut-off frequency was set at
12 kHz (fixed setting) for the Pf4001 and 22 kHz (selectable setting) for
the moorLAB. The analogue outputs of both monitors were connected to an
analogue-to-digital acquisition card (PC-LPM-16/PnP, National Instruments
Co., Austin, USA) sampling at a rate of 40 Hz with 12 bit of resolution. A
recording program was developed using Labview v5.1 (National Instru-
ments Co.) running on a personal computer.

The probe used on the dorsum of the foot was straight and cylindrical with
one central fibre surrounded by six fibres hexagonally distributed (fig. 4.1
left). All the fibres had a core diameter of 0.125 mm and a separation be-
tween fibres (centre-to-centre) of 0.25 mm. These six fibres had inde-
pendent connectors, therefore, we could do any combination of source and
detection fibres. Only one fibre was used as illuminating fibre (L, fig. 4.1) and was connected to the moorLab server’s laser. Two of the fibres located at 0.25 mm from the illuminating fibre were respectively plugged into the first Pf4001 detector (P1) and the other to the first moorLAB detector (M1). The only fibre at 0.50 mm from the illuminating fibre was plugged into the first moorLab satellite’s detector (M2). This probe was chosen because the fibre distances and fibre dimensions are in the range of what is delivered with these monitors in general.

The second probe, designed for measurements on the pulp of the hallux [16], was included in this study for comparison purposes. It was a 90°-angled probe with nine detection fibres and one illuminating fibre, all with a core diameter of 0.125 mm (fig. 4.1 right). The illuminating fibre was at the centre of a circle formed by the tips of the eight detection fibres, having a radius of 1.0 mm. Another detection fibre was located at 0.2 mm from the illuminating fibre. Only one cord came out of the probe, and at the distal edge, it ended in three sub-wires carrying respectively the 0.2 mm detector fibre (M3), the illuminating fibre and the eight 1.0 mm detection fibres (M4).

The monitors were calibrated using the probes and connections shown in Figure 4.1 and using the calibration liquid (motility standard) provided by the respective manufacturer, and following each manufacture’s calibration instructions. The calibration was performed under low ambient light conditions and at a room temperature of 22 ±1 °C. The resulting flux signals, from the respective motility standard, were 250 perfusion units (PU) for the Pf4001, and 220 PU for each channel of the moorLAB. After the initial calibration, the flux output signal was regularly checked before each measurement against the own motility standard. In case the obtained flux signal changed more than ±50 PU from the above calibration values, a new calibration was performed following the above procedure.

Arterial occlusion was performed using an 18-cm wide pneumatic cuff (CC17, D. E. Hokanson Inc., Bellevue, USA) attached around the thigh of the measured leg. The cuff was driven by a rapid cuff inflator system (E-20 rapid cuff inflator and AG-101 air source, D.E. Hokanson) to quickly inflate/deflate the cuff.

4.2.2 Test subjects

Approval for this study was obtained from the medical ethical committee of the University Medical Centre Groningen. Thirty subjects of a comparable age participated in this study and were distributed in three groups:
• PAOD: Ten subjects of type II-III Fontaine class peripheral arterial obstructive disease (with claudication or rest pain, and no critical limb ischemia). PAOD was documented by an ABI (ankle-brachial index) < 0.9, and/or Doppler ultrasound or angiographic studies confirming a severe stenosis or occlusion. Most of the patients were participants in a so-called walking training program. The mean age was 61 ± 9 years (range 49-76 years, six men).

• DM: Ten subjects with diabetes mellitus (type 1 and type 2) without symptoms of PAOD. The mean age was 52 ± 7 years (range 43-63 years, seven men). Diabetes had been diagnosed using conventional ADA criteria [1].

• Controls: Ten healthy subjects with no clinical symptoms of vascular disease. The mean age was 55 ± 8 years (range 46-68 years, 10 men).

Subjects with a history of congestive heart failure were excluded. Furthermore, drugs with vasoactive effects were not allowed in the hours before the measurement.

4.2.3 Test procedures

The subjects comfortably rested on a bed in supine position for 30 min prior to starting the recording. During this period, the two probes were attached to the right foot using double-sided adhesive rings. The straight probe was placed on the dorsum of the foot between the 2nd and 3rd metatarsals using a flat-circular probe plastic holder. The other probe was placed on the pulp of the big toe using a concave-circular silicon rubber holder. The brachial arterial pressure was measured and noted.

The recording session lasted 33 min without stops, and had three stages. The first stage lasted 15 min to record a stable resting flux reference signal. This was followed by an arterial occlusion by inflating the cuff 30 mm Hg suprasystolic for a maximum of 3 minutes. After the cuff deflation, the post-occlusive reactive hyperaemia (PORH) was recorded for up to 15 minutes.

4.2.4 Signal processing

The flux values measured were converted from Volts to perfusion units (10 mV = 1 PU), and averaged over one second to eliminate the heartbeats. The Biological Zero (BZ) [25] was then calculated by averaging the flux signal from the most representative excerpt of the occlusion period, as
Figure 4.1. Optical-fibre layout of the two skin probes used in this study.
this flux signal often presented relatively large signal fluctuations. The BZ value was then subtracted from every flux data value. Next, several parameters were calculated as follows:

- **Resting flux** (RF) was defined as the mean flux calculated from the longest signal excerpt free of artefacts, and representing the pre-occlusion period.

- The **time to resting flux** \( (t_{RF}) \) was defined as the time when the first flux data-point was higher or equal to the RF value.

- The flux signal was averaged even more using a 21-seconds moving average to obtain the following properties:

- The **time to maximum flux** \( (t_{MF}) \) and **maximum flux** (MF) values during the PORH curve were determined at the highest flux value after the cuff release.

- The **time of half recovery** \( (t_{HR}) \) was obtained by calculating first the half recovery flux (HRF) value defined as: \( HRF = (MF+RF)/2 \). Then, the \( t_{HR} \) value was obtained from the first flux data-point lower or equal to HRF.

- The **flux ratio** was defined as the ratio between MF and RF. This parameter shows the relative increase of flux and give an idea of the remaining reserve capacity of the microvasculature.

- The **fibre flux ratio** is the ratio between flux values obtained at two fibre separations during resting flux conditions. These were calculated only from the probes of the Moor monitor because it measured simultaneously with different fibre separations.

4.2.5 Data analysis

The comparison of data from different channels was performed for RF, MF, and Flux-Ratio values using scatter plots for each of the following channel pairs: M1-M2, M1-P1, M2-P1, and M3-M4. A second order curve was fit through the origin to calculate the \( R^2 \) value.

Summary statistics were calculated and the values were checked for normality. Because not all data were normal, the decimal logarithm was calculated to meet the normality requirements for using parametric tests. Cases with missing values were excluded per test. For every channel, the t-test was used to check the clinical value of each parameter to distinguish the patient groups. For every group, the parameters were compared for the
above mentioned channel pairs by using the paired samples t-test. All the statistical tests were two-tailed and a p < 0.05 was considered significant. The statistical analysis was performed using SPSS version 11.

4.3 Results

Comparison of patient groups and Controls

4.3.1 Comparison of fluxes

The flux signals from the toe were much larger than those from the dorsum. Large flux spikes were often observed from the dorsum signals, which sometimes complicated the graphical analysis of the tracing. In a few cases, signal artefacts occurred during the occlusion, however in most cases these were not accompanied by visible foot movements. Because of the large signals recorded from the toe, fewer artefacts were observed at this location.

In the dorsum as well as on the toe, none of the RF values for any monitor as well as for any fibre separation configuration were significantly different for the patient groups compared to the control group (Table 4.1). This was also true for the values of MF.

Furthermore, the comparison of the ratio between RF and MF fluxes, the Flux Ratio, did not show any significant difference between the Controls and the patient groups (Table 4.1) for any of the channels at both foot locations.

Finally, the ratio between RF values for smaller and larger fibre distances, the Fibre-Flux Ratio, was investigated for the Moor LDPM. For the dorsum as well as for the toe, the Fibre-Flux Ratio results were the same, and thus not significantly different for each group. The Fibre-Flux Ratio of the toe had a smaller value than that of the dorsum (Table 4.1).

4.3.2 Comparison of time-parameters

In three cases, $t_{RF}$ could not be calculated for the Flux signal (1 Control, and 2 PAOD) due to movement artefacts at cuff-release time. In the dorsum, $t_{RF}$ yielded significant differences ($p < 0.01$) between PAOD patients and Controls in all three channels, whereas between DM patients and Controls only significant differences ($p < 0.05$) were found for the Perimed LDPM, P1 (Table 4.1). On the toe, no significant differences were found between Controls and patient groups for $t_{RF}$.
For \( t_{MF} \) the results at toe and dorsum differed. At the dorsum, the Perimed LDPM (P1) was the only device that significantly discriminated between PAOD and Controls (p < 0.05). Both toe channels showed significant differences between PAOD and Controls for \( t_{MF} \) only, with p < 0.02 at 0.2-mm fibre separation (M4) and p < 0.04 at 1.0-mm fibre separation (M3).

For \( t_{HR} \) only significant differences were found for P1 between PAOD and Controls (p = 0.049).

**Comparison among channels**

### 4.3.3 Flux differences between channels at the same foot location

In the control group, significant differences were found for the RF values for the following channel comparisons: M1-M2 (p = 0.02), M1-P1 (p = 0.05), and M3-M4 (p < 0.001). For the PAOD patients, significant differences were found for M1-M2 (p = 0.001), M2-P1 (p < 0.05), and M3-M4 (p < 0.001). For the DM patients, the significantly different channel comparisons were M1-M2 (p < 0.001), M2-P1 (p < 0.01), and M3-M4 (p < 0.001).

**Table 4.1. Summary of the parameters calculated in this study. The parameters are: average resting flux (RF), time to RF \((t_{RF})\), maximum flux (MF), time to MF \((t_{MF})\), time of half recovery \((t_{HR})\), the flux ratio \((MF/RF)\), and the fibre-flux ratio. Values as mean ± SD.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dorsum Probe</th>
<th>Hallux Probe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moor 0.25 (ch. M1)</td>
<td>Moor 0.5 (ch. M2)</td>
</tr>
<tr>
<td>RF (PU)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.4 ±11.7</td>
<td>11.6 ±10.6</td>
</tr>
<tr>
<td>PAOD</td>
<td>8.5 ±7.3</td>
<td>12.8 ±9.9</td>
</tr>
<tr>
<td>Control</td>
<td>3.1 ±3.9</td>
<td>3.2 ±3.8</td>
</tr>
<tr>
<td>DM</td>
<td>15.2 ±20.3</td>
<td>15.6 ±21.0</td>
</tr>
<tr>
<td>PAOD</td>
<td>24.3 ±19.2</td>
<td>24.4 ±19.1</td>
</tr>
<tr>
<td>Control</td>
<td>37.0 ±13.9</td>
<td>37.0 ±13.6</td>
</tr>
<tr>
<td>DM</td>
<td>45.3 ±31.3</td>
<td>49.4 ±40.9</td>
</tr>
<tr>
<td>Tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RF (PU)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>82.1 ±72.2</td>
<td>77.6 ±65.0</td>
</tr>
<tr>
<td>PAOD</td>
<td>24.2 ±20.6</td>
<td>37.9 ±26.6</td>
</tr>
<tr>
<td>Control</td>
<td>19.4 ±11.8</td>
<td>31.2 ±17.8</td>
</tr>
<tr>
<td>DM</td>
<td>23.0 ±16.7</td>
<td>32.9 ±21.2</td>
</tr>
<tr>
<td>Tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RF (PU)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>70.8 ±13.0</td>
<td>73.6 ±15.8</td>
</tr>
<tr>
<td>PAOD</td>
<td>83.7 ±39.2</td>
<td>90.4 ±42.9</td>
</tr>
<tr>
<td>Control</td>
<td>122.1 ±71.8</td>
<td>129.3 ±79.4</td>
</tr>
<tr>
<td>DM</td>
<td>4.49 ±3.92</td>
<td>4.68 ±4.72</td>
</tr>
<tr>
<td>Tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flux ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.14 ±1.35</td>
<td>2.90 ±1.05</td>
</tr>
<tr>
<td>PAOD</td>
<td>0.63 ±0.22</td>
<td>-</td>
</tr>
<tr>
<td>Fibre Flux ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.61 ±0.07</td>
<td>-</td>
</tr>
<tr>
<td>PAOD</td>
<td>0.65 ±0.20</td>
<td>-</td>
</tr>
</tbody>
</table>
In other words, the only non-significant differences occurred in comparisons with different instruments, M1-P1 in the patients with PAOD or DM and M2-P1 in the Controls.

For the maximum flux (MF) in the dorsum, the M2 channel showed the highest values, whereas the M1 and P1 channels gave lower but similar MF values (Table 4.1). In the Control and PAOD groups significantly different MF-values were found only for the comparison of the M1-M2 and M3-M4 channels (p < 0.01), whereas the other two comparisons were not (M1-P1 and M2-P1). The DM group presented significant differences for the pairs M1-M2 (p < 0.001), M2-P1 (p < 0.02), and M3-M4 (p < 0.001).

At the dorsum, the flux parameters (RF and MF) were typically lower for the M1 channel than for M2. This can also be seen from the fibre-flux ratio values in Table 4.1, which always were around 0.6 in every group. Furthermore, for the Moor at 0.50 mm (M2) and the Perimed at 0.25 mm (P1) channels appeared to give similar RF values (Table 4.1). Figure 4.2A gives an example from a patient with DM. However, similar RF values were not always found, as in a few cases RF and MF were very similar for M1 and M2.

Figure 4.2B shows the large difference between the M3 and M4 flux signals recorded from the toe, giving lower output for M4 than for M3 in all cases. The mean values of the Fibre-Flux Ratio for the M3-M4 channels were between 0.3 and 0.4.

<table>
<thead>
<tr>
<th></th>
<th>Dorsum Probes</th>
<th>Hallux Probe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M1-M2</td>
<td>M1-P1</td>
</tr>
<tr>
<td><strong>RF</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All Groups</td>
<td>0.935</td>
<td>0.827</td>
</tr>
<tr>
<td>Control</td>
<td>0.931</td>
<td>0.798</td>
</tr>
<tr>
<td>DM</td>
<td>0.987</td>
<td>0.983</td>
</tr>
<tr>
<td>PAOD</td>
<td>0.925</td>
<td>0.756</td>
</tr>
<tr>
<td><strong>MF</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All Groups</td>
<td>0.666</td>
<td>0.836</td>
</tr>
<tr>
<td>Control</td>
<td>0.497</td>
<td>0.843</td>
</tr>
<tr>
<td>DM</td>
<td>0.846</td>
<td>0.962</td>
</tr>
<tr>
<td>PAOD</td>
<td>0.802</td>
<td>0.772</td>
</tr>
<tr>
<td><strong>Flux Ratio</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All Groups</td>
<td>0.983</td>
<td>0.983</td>
</tr>
<tr>
<td>Control</td>
<td>0.987</td>
<td>0.985</td>
</tr>
<tr>
<td>DM</td>
<td>0.994</td>
<td>0.984</td>
</tr>
<tr>
<td>PAOD</td>
<td>0.993</td>
<td>0.990</td>
</tr>
</tbody>
</table>

*Table 4.2. Comparison of $R^2$ values calculated between several pairs of channels obtained by fitting a straight line through the origin.*
The correlation between values of RF, MF, and Flux Ratio from different fibre distances or instruments were each compared by calculating the correlation coefficients from XY-plots. Table 4.2 gives results for all investigated channel pairs. The correlations between channels with the same instrument for RF were higher for the foot than for the toe, whereas the situation for MF was opposite. For correlations of RF and MF fluxes between different instruments for the foot, the M2-P1 comparisons gave higher correlations than the M1-P1 comparisons, except for the DM group.

Figure 4.2. Example of Flux tracings obtained at the dorsum of the foot (A) and at the toe (B) of a DM patient. The flux values shown are corrected for the biological zero.
Results of comparisons for RF and MF values are depicted in Figure 4.3 for all dorsum channels. Second-order fitting curves were used to show the trend of M1 and P1 compared to M2. A linear relation was observed for the comparison of the values of the M1 and M2 channels, whereas the comparison between P1 and M2 channels lost linearity at high flux values.

The Flux Ratio values were very similar for both devices (see Table 4.1). The correlation coefficients $R^2$ for the Flux Ratio were high for all comparisons, including the comparisons between P1-M2 and P1-M1, the comparisons between different instruments (Table 4.2).

4.3.4 Comparison of time parameters at the same foot location

In dorsum and toe, both instruments showed similar reaction times in the PORH responses (Table 4.1). Figure 4.4 compares the time to RF ($t_{RF}$) values of all cases for the three dorsum channels. In the dorsum only, the paired samples test for $t_{RF}$ resulted in significantly differences between P1 and M1 in the DM group only ($p = 0.036$).

4.3.5 Comparison between dorsum and toe

In all cases, the RF and MF values recorded from the channel M4 (0.20 mm) were much higher than those from channel M1 (0.25 mm). In a XY plot (not shown) for RF a value of $R^2 = 0.592$, for MF of $R^2 = 0.766$, and for the Flux Ratio (MF/RF) of $R^2 = 0.49$ were found. The $R^2$ for the time parameters were $R^2 = 0.716$ for $t_{RF}$, $R^2 = 0.83$ for $t_{MF}$, and for $t_{HR}$ $R^2 = 0.85$. Only for the control group, the $t_{RF}$ values were very significantly different between M1 and M4 ($p < 0.01$).

4.4 Discussion and Conclusions

4.4.1 Differences between the instruments

The present study was performed to support the comparison of results obtained with LDPM systems supplied by different manufacturers. As the different motility standards that are supplied by each manufacturer lead to different calibrations of the instruments, it was decided to calibrate each monitor using its respective manufacturer’s calibration kit. In this way, the monitors were calibrated in a similar way as any other user could have performed with only one of these devices. It should be noted that studies with more interests in future standardisation or comparing the signal processing of the instruments may choose for a common calibration kit [22].

The two monitors that were used in the present study showed a slight loss of linearity in the Perimed monitor compared to the Moor (fig. 4.3). This
effect may be caused by the lower frequency cut-off of 12 kHz of the Perimed compared to the 22 kHz used in Moor, which might cause loss of linearity at high fluxes. High flux values may occur during some of the manoeuvres performed in the clinic, such as during the post-occlusive reactive hyperaemia. Another explanation for the loss in linearity might be the adapted data processing of the Perimed LDPM to correct for non-linear behaviour at high levels of the red cell concentration in tissue [18], which may also take place during the post-occlusive reactive hyperaemia.

At a fibre distance of 0.25 mm the mean values for RF per subject for Controls as obtained at the dorsum with the Perimed system were significantly larger than those with the Moor system. The ratio between these values may give a constant factor for comparison of results between instruments for this fibre distance. This difference between the Perimed and Moor devices at 0.25 mm was not significant for RF in patients and for measurements of MF.

Because of this lack of significance, the question may be posed whether this may be due to local differences in the vascular structure under the fibres. Because of the higher correlations in most cases for M1–M2, with similar differences in vascular bed compared to M1-P1, this is not likely. Therefore, the observed differences are more likely due to the differences between the instruments that were discussed above than to differences in the vascular bed under the fibres. Nevertheless, to avoid influences of differences in the vascular bed below the probe, a more idealised comparison of LDPM monitors may be that of Petoukhova et al., who used a single detection fibre and a fibre splitter to feed three different monitors from an unique detection fibre [22].

Furthermore, it was often observed that the M2 and P1 channels gave similar RF values, mostly for the control group, and also showed a better correlation than M1 and P1, which was comparable with the correlation between the M1 and M2 signals. Despite of the different fibre distances, these results suggest that the flux signals from the M2 and P1 channels are better comparable than those between M1 and P1. As both the average length of the photon path and the penetration depth of the detected light increase with the larger fibre distances [8,9,11], more Doppler shifts will occur for larger fibre distances. Thus, the frequency range of the detected signal will increase in that case. This may imply that the moorLab measuring RF with the higher frequency range at 0.5 mm and the Perimed signal at 0.25 mm suffer from similar restrictions in the wavelength band. However, this is probably not true, as the correlation between M1 and M2 signals was high. Unknown mechanisms in the physics of perfusion and blood properties may be responsible for the differences between the instruments.
Figure 4.3. The resting flux and maximum flux values of all the subjects are here compared per detection channel against M2 channel. A polynomial line was fit to the M2-M1 channels (open circles) and to the M2-P1 channels (close circles). The dashed line is the identity line of the M2 channel (Moor 0.50 mm).

Figure 4.4. The $t_{RF}$ values of all subjects from the dorsum were compared between the M2-M1 channels (crosses) and the M2-P1 channels (open circles). The dashed line is the identity line for the M2 channel (Moor 0.50 mm).
4.4.2 Effect of fibre distance

Laser Doppler perfusion monitors are available with various fibre separations. Therefore, we studied its effect on the measured flux. The Fibre Flux Ratio tells that the influence of the fibre separation as measured with the moorLab system on the dorsum of the foot as well as on the toe is rather constant and does not vary with the subject groups. In an earlier study on the RF-Fibre Flux Ratio, a significant difference in the fibre RF-Flux Ratio between Patients and Controls was found [16]. Such a difference was not found in the present study. Those results probably have to be ascribed to the lower age of the control group in that study (mean age 29.1 years, range 23-47 years), which showed an RF-Fibre Flux Ratio of 0.46 ±0.08 on the toe with the same instrumentation and toe probe as used in the present study. Therefore, it is suggested that correction of the flux for the applied fibre distance probably is a function of age, with neglectable differences between patient groups and controls. This is supported by the finding that the mean values of the Fibre-Flux Ratio per patient group from the present data also increase with the mean age of the patient group.

Values of the RF-Fibre-Flux Ratio are available for the toe and for the dorsum of the foot and can thus be applied to all groups to adapt results obtained at intermediate fibre distances. The fact that the values of the RF Fibre Flux Ratio at the dorsum are closer to 1.0 than those at the toe has to be ascribed to the smaller relative difference of fibre distances at the toe. To compare your results obtained at distance \( r \), we suggest to compare to the flux results at a given distance \( r_0 \) in Table 4.1 multiplied by \((r/r_0)^{0.66}\). It should be noted that this correction equation might depend on the distribution of perfusion under the probe as a function of depth. Furthermore, usage of this factor may be restricted to results with the moorLab system, as results depend on the motility standard that is used during calibration. For the case that the moorLab would be calibrated with Perimed’s motility standard, flux values as well as the fibre flux ratio would give different results. Also the correction equation given above would lead to different results (unpublished results). For comparison with moorLab results, the flux values in Table 1 obtained with the moorLab approximately have to be multiplied by \( 0.88 \cdot r^{0.22} \), with \( r \) in mm. In combination with the correction for fibre distance dependence, for the results given above, this gives the distance dependence of results that would have been found after calibration in Perimed motility standard: \((r/r_0)^{0.88}\).

The Flux Ratio (MF/RF) showed similar values for all channels and high correlations, especially for the dorsum of the foot. It should be noted that the similar results of RF/MF, which were obtained when comparing instruments or results at different fibre distances at the same probe location, are not
changed by the application of a correction factor between instruments to both RF and MF, nor by the use of a correction factor for fibre distance.

4.4.3 Patient groups

With respect to clinical applicability of our data, none of the flux values in Table 4.1, nor any Flux Ratio or Fibre Flux Ratio showed significant differences between the Patient groups and Controls. It was remarkable that the patient group with Diabetes Mellitus showed the highest correlation between channels for RF and MF. Significant differences in the Flux Ratio may be found in a study with larger groups or in patients in a more advanced stage of disease.

The dorsum channels M1, M2, and P1 showed a high correlation for the flux values, independently of the fibre distance correction as mentioned above. It shows that the calculation of time parameters from the flux measurement at the dorsum of the foot does not depend very much on the fibre distance. On the other hand, it is remarkable that the correlation coefficients for M1-M2 in Table 4.2 were much lower for the Controls than for patients with DM. Perhaps, microvascular dysfunction in Diabetes Mellitus is associated with a loss in local perfusion variability.

Not surprisingly, the comparison of measurements between the dorsum of the foot and the pulp of the hallux were poorly correlated, even though only data from the same device were compared. This suggests that the flux signal from the toe mostly resulted from the thermoregulatory part of the microcirculation that is much more developed than in the dorsum of the foot.

4.4.4 Time-parameters

From this study, it was observed that both devices showed equal reaction times to changes in the tissue perfusion on a common foot location. The time parameters calculated from the PORH response correlated very well among the dorsum channels and also showed very high $R^2$, as shown in Figure 4.4. Time parameters were shown to be of high importance, as only the time parameters could distinguish between patients and controls.

The measurement of $t_{RF}$, when measured at the dorsum of the foot, were shown to be superior compared to the other time characteristics in distinguishing between patients with POAD and Control subjects. Similar results were found in a previous study with a larger group of subjects which only used Perimed perfusion monitors [17]. The present study showed that the results of $t_{RF}$ did not depend on the instrument nor on the fibre distance and allowed a clear separation between Controls and PAOD patients. The
fact that this parameter did not differ between the instruments nor on the fibre distance makes it a potentially attractive parameter for clinical use.

The differences in the time parameters observed among the groups between in the toe and the dorsum are remarkable. For the PAOD and DM groups, the time to resting flux (t_{RF}) values were the same on the dorsum channels and the channel M4, respectively. For the control group, the t_{RF} was the same for the three channels on the dorsum (M1, M2, P1) but slightly larger on the toe (M3, M4). As no significant results were observed at the toe between t_{RF} in Controls and patients with PAOD, this study concludes that the dorsum of the foot is to be preferred to measure t_{RF}.

In conclusion, this study showed that the Pf4001 and the moorLab monitors deliver flux signals as a result of simultaneously assessing similarly perfused skin tissue. Introduction of a correction factor will improve the comparability, although the present results showed that a full conversion will not be easy to find. Comparison of relative flux values, such as the flux ratio, was practically independent of the instrument or fibre distance. Comparison of other flux results obtained at various fibre distances was shown to be possible with some restrictions. However, in our results the flux values were of little clinical value. In contrast, the reaction time t_{RF} could distinguish between Controls and PAOD patients, and may therefore be of clinical value. It was approximately the same for both monitors, allowing the comparison of LDPM measurements between different instruments and fibre separations in the described conditions. Overall, it is concluded that LDPM values based on characteristic times and relative changes in the microcirculatory flow seem to be more useful for comparisons than direct measures of the flux.

4.5 References


27. Zijlstra WG, Buursma A, Meeuwen-van-der-Roest WP. Absorption spectra of human feto-
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