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Assessed With Velocity-Resolved Quantitative  
Laser Doppler Flowmetry**

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# Reduced arterio-venous shunting capacity after local heating and redistribution of baseline skin blood flow in type 2 diabetes assessed with velocity-resolved quantitative laser Doppler flowmetry

Running title: Reduced arterio-venous shunting capacity

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## ***Abstract***

*Objective:* To compare the microcirculatory velocity distribution in type 2 diabetes patients (DM) and non-diabetic controls (ND) at baseline and after local heating.

*Research design and methods:* The skin blood flow response to local heating (44°C for 20 min) was assessed in 28 DM patients and 29 ND subjects using a new velocity-resolved quantitative laser Doppler flowmetry technique (qLDF). The qLDF estimates red blood cell (RBC) perfusion (velocity times concentration), in a physiological relevant unit (g RBC / 100 g tissue × mm/s) in a fixed output volume, separated into three velocity regions,  $v < 1$  mm/s, 1 - 10 mm/s, and  $v > 10$  mm/s.

*Results:* The increased blood flow occurs in vessels with a velocity above 1 mm/s. A significantly lower response in qLDF total perfusion was found in DM than in ND after heat provocation, due to less high-velocity blood flow ( $v > 10$  mm/s). The RBC concentration in DM increased 7-fold for  $v$  between 1-10 mm/s and 15-fold for  $v > 10$  mm/s, while no significant increase was found for  $v < 1$  mm/s. The mean velocity increased from 0.94 to 7.3 mm/s for DM and from 0.83 to 9.7 mm/s in ND.

*Conclusions:* The perfusion increase occurs in larger shunting vessels and not as an increase in capillary flow. Baseline DM data indicated a redistribution of flow to higher velocity regions, associated with longer DM duration. A lower perfusion was associated with a higher BMI and a lower toe-to-brachial systolic blood pressure ratio.

## ***Introduction***

Laser Doppler flowmetry (LDF) has frequently been used to measure blood flow abnormalities in diabetes mellitus (DM). (1-5) A reduced vascular response to local heating, due to loss of the active neurogenic vasodilation, has been observed in type 1 and 2 DM patients using LDF, (1, 6) and due to loss in endothelium NO synthesis in type 2 DM patients (7). Another microvascular abnormality has also been suggested – an increased flow through the arteriovenous (AV) shunts resulting in a reduced capillary flow. (8-12) This is most evident during provocations such as postocclusive

reactive hyperemia and in patients with severe neuropathy, but it can also be observed at rest in DM patients without complications. (11)

While differences in blood flow between DM and controls have been found using LDF during heating and other provocation protocols, results during baseline conditions have been more conflicting. (6) Studies have reported a lower blood flow in DM compared with controls (3), no difference (1, 4), and even tendencies towards a higher blood flow in DM. (11) The conflicting results are partly explained by the different measurement positions used, where differences have been seen especially between glabrous (palms, soles etc.) and nonglabrous (hairy) skin. (6) While authors most often specify the measurement position in detail, unfortunately it is common that the specification of the LDF instrumentation used is omitted, in terms of operating wavelength and probe type/source-detector separation. The measurement depth is highly dependent on these two properties, especially the latter (13), and therefore some of the discrepancies in the reported results may be explained by the differences in instrumentation.

Local warming of skin causes a biphasic increase in skin blood flow as measured by LDF. The initial increase in blood flow that sometimes temporarily declines is mediated by a C-fiber axon reflex, while the second prolonged phase requires nitric oxide (NO). (14, 15) These and other studies use LDF for elucidating NO synthase mechanisms (5, 7, 16, 17), for quantification of abnormalities in the endothelial dependent NO vasodilation (18) and other microvascular research issues. (19, 20)

Studying the microcirculation in a clinical setting using LDF may be difficult, since the method only provides relative measures on skin blood flow. (14) Large local spatial and temporal variations in blood flow have also been reported. (21) Furthermore, measures are given for an unknown measurement volume that varies not only with instrumentation but also with the optical properties at the measurement site and with variations in the blood flow itself. (13) This further obstructs the physiological interpretation of the results.

We have developed a new LDF signal analysis based on simulations of light transport in an adaptive mathematical model of tissue. As the perfusion estimates achieved with this method are

presented in physiologically relevant units (g red blood cells (RBC)/100 g tissue  $\times$  mm/s) in a given output volume (3 mm<sup>3</sup>), the method is called quantitative LDF (qLDF). Besides the benefits of a constant output volume and physiologically relevant units, the method also provides a means of differentiating the blood flow into various flow velocity regions. (22) This velocity differentiation can potentially be used to associate differences in the velocity distribution to specific vessel types as large vessels have normally a much higher blood flow velocity than smaller vessels.

The aim of the present study was to compare the velocity distribution of the microcirculatory blood flow between patients with diabetes type 2 and non-diabetes controls during baseline and local warming. To our knowledge, this has previously not been possible, and the differences in the velocity distribution can be used to draw conclusions about the type of vessels that are mainly involved in the pathological disturbances of the microcirculation in diabetes type 2.

### ***Research Design and Methods***

The patients included in this study are part of a larger study called CARDIPP (Cardiovascular Risk Factors in Patients with Diabetes – a Prospective Study in Primary Care) that was launched in 2005 with the aim of identifying markers for cardiovascular disease to facilitate earlier and individually adjusted intervention, in middle aged patients with type 2 diabetes. The non-diabetes controls were part of a parallel control study called CAREFUL. Within the CARDIPP and CAREFUL studies, parameters such as height, weight, and blood pressure were measured. The investigation included medical history, also covering data on diabetes duration and ongoing medication. Blood specimens were drawn in the morning after a 10 hour overnight fast. Aortic pulse wave velocity (PWV) was measured with applanation tonometry (Sphygmocor<sup>®</sup>) over the carotid and femoral arteries as an indicator of arterial stiffness. Intima Media thickness of the carotid arteries (IMT) was evaluated using a B-mode ultrasound, as a measure of subclinical atherosclerosis. The toe-brachial index (TBI; toe systolic blood pressure-to brachial systolic blood pressure ratio) was used as a measure of

peripheral arterial disease. For more details about these studies, see References (23, 24). The protocol was approved by the local ethical committee (D.no. M26-05).

Based on accessibility, a small number of the subjects included in CARDIPP and CAREFUL were asked to join the extended study where also microcirculatory parameters in the foot were measured using a modified LDF instrument (Perimed AB, Järfälla, Sweden). Recordings were made using a custom made probe with one light emitting fiber placed in the center, one light collecting fiber placed 0.25 mm (center-to-center distance) from the light emitting fiber and another 11 light collecting fibers placed in a circle  $1.2 \pm 0.1$  mm from the light emitting fiber. All fibers were made of silica with a 0.125 mm diameter and  $NA = 0.37$ , and the light source was a 780 nm laser diode. Backscattered light collected at the two source-detector distances was separately detected and sampled at 50 kHz for later analysis. The measurement probe was placed in a thermostatic probe holder (PF 450, Perimed AB, Järfälla, Sweden) that was able to adjust the skin temperature and assured a minimum pressure contact between the probe tip and the examined skin. All measurements were performed on the dorsum of one foot 3-6 cm proximal of the toes between the metatarsals avoiding visible vessels. (1) The recordings lasted for 25 minutes. In the beginning of the recording the probe holder was heated to 32°C, defined as the baseline, and after approximately 5 minutes, the temperature was increased to 44°C, which lasted throughout the rest of the measurement. All measurements were performed in a room keeping a temperature of 23-25°C and the subjects were placed in the supine position. The subjects were acclimatized in the room for at least 15 minutes prior to the measurement. The measurements were done at approximately the same time of day for the two groups, at 11.58 am (1:51 h) for ND and at 11.13 am (1:32 h) for DM (mean (SD)). The extended protocol was approved by the local ethical committee (D.no. M26-05 T41-08).

From the recorded signals, Doppler power spectra were calculated, calibrated, and averaged in 5 second intervals for the two different source-detector separations. (22, 25) Conventional LDF perfusion measures (26) were calculated for both separations from the calculated and averaged Doppler power spectra. These measures are given in arbitrary units with unknown and non-constant sampling volumes. Besides the conventional perfusion measures, the quantitative LDF method was

used to estimate the perfusion in a physiological relevant unit ( $\text{g RBC} / 100 \text{ g tissue} \times \text{mm/s}$ ). The value is relevant for an output volume of a  $3 \text{ mm}^3$  half sphere, i.e. within a 1.13 mm radius from the emitting fiber. The perfusion measure is divided into three velocity regions,  $v < 1 \text{ mm/s}$ ,  $1 < v < 10 \text{ mm/s}$ , and  $v > 10 \text{ mm/s}$ . Furthermore, the total perfusion is also presented (sum of all three regions). The method, presented in (22), is based on a model adaption of the Doppler power spectra to the measured spectra, at the two source-detector separations simultaneously.

Blood perfusion was measured in 28 DM patients and in 29 non-diabetes controls (ND). Three DM and three ND were excluded due to varicose veins, one DM was excluded due to a skin surface temperature above  $32 \text{ }^\circ\text{C}$ , one ND was excluded due to chronic obstructive pulmonary disease, and one ND was excluded due to suspected poor contact between the probe and the skin. In two of the measurements, one DM and one ND, the method was not able to find a model that matched the measured spectra ( $\chi^2 > 0.5$ ) and they were therefore excluded. The remaining DM patients and ND controls are described in Table 1. Among the 23 included DM patients, seven were treated with diet and exercise only, 13 were also treated with oral hypoglycemic agents but no insulin, and three were treated with insulin. Eleven subjects were on metformin, two on sulphonylurea, two on thiazolidinediones and one subject was treated with a DPPP IV-inhibitor. Three individuals did not take any antihypertensive medication and the most frequently used antihypertensive drug was ACE/ARB (14 subjects), followed by eight subjects on beta-blockers, five were on diuretics and four on calcium channel blockers. Twenty subjects were treated with statins. Four subjects were current smokers and 12 were ex-smokers. Microalbuminuria, defined as albumin/creatinine ratio  $> 30 \text{ } \mu\text{g}/\text{mg}$ , was present in one subject. History of cardiovascular disease was prevalent in three subjects. The baseline measurement was excluded for 3 DM patients due to a too short acclimatization period (less than 15 minutes).

Statistical comparisons between DM and ND were performed using the non-parametric Mann-Whitney U-test. Bivariate correlations were evaluated using Pearson's product-moment analysis. Outlier data were excluded in the correlation analysis, using an upper limit of the mean + 3 SD of DM data. A p-value below 0.05 was considered significant.

## *Results*

Examples of the qLDF perfusion measures during the heat provocation are shown in Figure 1. In Figure 1a, typical behavior of the perfusion in the three different velocity regions is shown. Only a small increase was found in the low-velocity region, while a larger increase occurred in the mid- and high-velocity regions. In Figure 1b, two typical heat responses of the total perfusion (all velocities) are shown. In the first case (Figure 1b; circles) the perfusion decreases after the initial heat induced increase, and shortly thereafter the perfusion increases again. In the second case (squares), this biphasic response is not visible and the perfusion quickly settles on a more or less constant level after the temperature rise. Both types of responses occurred in both DM patients and controls and were approximately equally common. In Figure 2, a comparison of the response of the qLDF perfusion estimate and the cLDF perfusion estimates at the two source-detector separations is shown. The cLDF perfusion estimates have been normalized so that they equal the qLDF perfusion estimate during baseline (the first five minutes). It can be seen that the heat response is much higher for the qLDF perfusion estimate than for the cLDF. On average for all 20 included DM patients (the three DM patients with too short acclimatization excluded), a 1065% increase was observed in the qLDF total perfusion, whereas the increase was 672% and 502% for the cLDF perfusion estimates at 0.25 and 1.2 mm source-detector separation, respectively. Corresponding increases for the 23 ND subjects were 1755%, 1046%, and 734%, respectively.

A summary of the cLDF and qLDF perfusion measures are given in Table 2. Values are given for baseline, i.e. the median during the first 5 minutes when the probe holder was heated to 32°C and for the plateau of the heat provocation response, i.e. the median of the last 5 minutes. No significant differences were found at baseline. For the plateau, significant differences between DM patients and ND subjects were found for cLDF using both source-detector separations (0.25 mm and 1.2 mm) and for qLDF total perfusion and for velocities above 10 mm/s. A similar analysis of the perfusion during the initial peak in the heat response, i.e. the median over two minutes starting two minutes after the heat onset, gave that both cLDF measures and qLDF total perfusion, were significantly lower for DM than for ND (data not presented).

Bivariate correlation analysis for diabetes patient data were performed at baseline and at 44°C for the two conventional LDF perfusion estimates, the quantitative perfusion in the different velocity regions and the total perfusion, the concentration of moving blood cells (Table 3) and their average velocity (Table 4), versus diabetic duration, HbA<sub>1c</sub> and TBI. Significant correlations were found at baseline for: duration versus the quantitative perfusion in the low-velocity region ( $R = -0.46, p < 0.05$ ), in the mid-velocity region ( $R = 0.48, p < 0.05$ ), the average velocity ( $R = 0.63, p < 0.01$ ) and the average concentration ( $R = -0.55, p < 0.05$ ). The relations between duration and qLDF data at baseline are presented in Figure 3. No significant correlations were found for HbA<sub>1c</sub> versus any of the perfusion estimates. Significant correlations were found after heat for: TBI versus the conventional perfusion at 1.2 mm separation ( $R = 0.42, p < 0.05$ ), the quantitative total perfusion ( $R = 0.42, p < 0.05$ ) and the average velocity ( $R = 0.46, p < 0.05$ ). The relations between TBI and qLDF data after heat are presented in Figure 4.

Bivariate correlation analysis for ND data were performed at baseline and after heat for the two conventional LDF perfusion estimates, the quantitative perfusion in the different velocity regions and the total perfusion, the concentration of moving blood cells and their average velocity, versus TBI. No significant correlations were found.

Bivariate correlations between the cLDF perfusions at both fiber distances and the total qLDF perfusion and BMI, LDL, glucose level and pulse wave velocity, respectively, were performed for DM and ND separately after heat provocation. No significant correlation was found in the ND group between any LDF perfusion measure and any clinical variable. For DM, LDL was related to cLDF perfusion at 0.25 mm separation ( $R = -0.49, p < 0.05$ ) and BMI was related or borderline related to three perfusion measures (cLDF perfusion at 0.25 mm fiber separation,  $R = -0.49, p < 0.05$ ; cLDF perfusion at 1.2 mm fiber separation,  $R = -0.40, p < 0.06$ ; qLDF total perfusion  $R = -0.41, p < 0.06$ ).

## *Discussion*

In about half of the measurements, both for the DM patients and ND subjects, a distinct biphasic heat response could be observed. The first part of this biphasic response is foremost related to the fast axon reflex, while the second part is related to the NO induced response. (14, 15) In the measurements where this biphasic response is not visible, the two phases are overlapping. The biphasic response becomes more evident when the heat increase is slower. (15) An attenuated response has previously been reported for both the axon reflex and the NO induced response in DM type 2 patients, (7, 27) and a significant difference between DM patients and controls in the increase from baseline to both the initial peak and the late plateau was also found in the current study. The level of the increase was similar to that reported by Colberg *et al.*, who used a similar protocol. (1)

Figure 2 shows an example of the perfusion response to heat for both the cLDF perfusion estimates at the two different source-detector separations and for the qLDF perfusion estimate. It illustrates that the relative increase from baseline to the late plateau was higher for the qLDF than for the cLDF estimates. The response for the 1.2 mm source-detector separation was weaker than for the 0.25 mm separation for the cLDF estimates. Actually, the increase for qLDF was higher than the increase in the cLDF for all measurements but one, and the increase for the long fiber separation was generally lower than the increase for the short one. This difference between the qLDF and cLDF perfusion estimates is due to the well-known non-linearity to the blood tissue fraction in cLDF perfusion, which is not present in qLDF perfusion (22). The non-linearity is not severe, nor does it have any great impact on the results in this study. However, when measuring on sites with higher blood tissue fraction than the dorsum of the foot, which has a relatively low blood tissue fraction, this may be of major importance. A method has previously been presented to compensate for the non-linearity in the cLDF perfusion estimates. (28) However, that method was designed and evaluated using plastic phantoms and its usefulness for *in vivo* measurements has been questioned by others (29, 30).

Table 2 shows that the high velocities are affected the most by the heat provocation and that they also differ the most between the DM patients and the ND subjects. In contrary, no significant increase is found in the perfusion for the lowest velocity region and no significant difference between the DM patients and the ND subjects is found in the two lowest velocity regions. Knowing that the thermoregulation involves mostly the larger vessels in the microcirculation where the velocity is generally higher, it is expected that the high velocities are affected the most by the heat provocation. The unchanged low velocity flow and the highly increased high velocity flow indicate that the increased flow due to the heat provocation is shunted from the artery to the vein side. The lower increase of the high velocity blood flow for DM patients compared to ND subjects thus strongly suggests that the maximal AV shunting capacity is reduced in DM patients. Furthermore, a decreasing perfusion response to heat was associated with increasing BMI and decreasing TBI, and similarly a decreasing average velocity with decreasing TBI. These relationships were observed only in DM patients but not in ND subjects. The clinical importance of these findings remains to be answered in the future.

Relatively large standard deviations were found for all perfusion estimates presented in Table 2, both at baseline and during the heat provocation. This may not only reflect intra-individual differences, but may also be a result from large spatial inhomogeneities in the microcirculation. (21) Although Colberg *et al.* argue that performing the measurements in a thermoneutral environment should reduce these inhomogeneities (1), which should be further reduced by the 32°C baseline level of the probe holder, the study would have benefited from measurements at multiple sites. For baseline measurements that must not exceed a couple of minutes, multiple measurement sites are recommended for future studies. Another limitation of this study is that we were not able to control for differences in antihypertensive medication between the two groups.

As stated in the introduction, results concerning the microvascular blood flow in DM patients in baseline have been contradictory. (1, 3, 4, 11) In this study, no significant differences were found between the DM patients and the control group (Table 2), but a correlation between baseline flow and diabetes duration was found (Figure 3). It is very interesting to observe that this correlation was

the opposite for the lowest and middle velocity regions, as resolved with the quantitative LDF method. The intriguing interpretation of this would be to relate the perfusion of the low velocity region ( $v < 1$  mm/s) to nutritive capillary flow, and the perfusion in the middle velocity region ( $1 < v < 10$  mm/s) to flow in arterioles, venules, and AV shunts. This interpretation is supported by the findings that the AV shunt flow is increased in DM at baseline resulting in a reduced nutritive capillary flow- (8-12) Although the maximal capacity of these shunting vessels is reduced.

### ***Conclusions***

By using a novel quantitative LDF method we have been able to compare the velocity distribution between DM patients and ND controls during baseline and after a local heat provocation. Our main findings include that (1) the heat provocation increases the blood flow for velocities over 1 mm/s, whereas blood flow at lower velocities is unchanged, (2) the reduced perfusion increase after local heating that is observed in DM compared to ND is found for velocities above 10 mm/s, and (3) a reduced low velocity flow ( $< 1$  mm/s) and increased mid velocity flow (1-10 mm/s) is related to diabetes duration at baseline conditions. The physiological interpretations of these results are that (1) the thermoregulation is a process involving primarily large, high velocity vessels and the increased flow is shunted from the artery to the vein side, (2) the maximal AV-shunting capacity is reduced in DM patients, and (3) a long diabetes duration is associated with a reduced nutritive capillary flow due to an increased AV-shunt flow at baseline conditions. All of this has previously been known or suspected but not proven by direct measurements on the microcirculation.

### ***Authors' contributions***

Ingemar Fredriksson: Writing the manuscript, Discussing study design and methods, Data collection, Data processing

Marcus Larsson: Discussing study design and methods, Data Collection, Discussing and reviewing the manuscript

Fredrik H. Nyström: Discussing study design and methods, Contributing with clinical data,  
Discussing and reviewing the manuscript

Toste Länne: Discussing study design and methods, Contributing with clinical data, Discussing and  
reviewing the manuscript

Carl J. Östgren: Discussing study design and methods, Contributing with clinical data, Discussing  
and reviewing the manuscript

Tomas Strömberg: Writing the manuscript, Discussing study design and methods, Data collection,  
Statistical data analysis

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## *References*

1. Colberg SR, Parson HK, Nunnold T, Herriott MT, Vinik AI, Effect of an 8-week resistance training program on cutaneous perfusion in type 2 diabetes. *Microvasc Res*, 2006. 71(2): p. 121-127.
2. Khan F, Elhadd TA, Greene SA, Belch JFF, Impaired skin microvascular function in children, adolescents, and young adults with type 1 diabetes. *Diabetes Care*, 2000. 23(2): p. 215-220.
3. Rendell M, Bamisedun O, Diabetic Cutaneous Microangiopathy. *Am J Med*, 1992. 93(6): p. 611-618.
4. Stansberry KB, Peppard HR, Babyak LM, Popp G, McNitt PM, Vinik AI, Primary nociceptive afferents mediate the blood flow dysfunction in non-glabrous (hairy) skin of type 2 diabetes - A new model for the pathogenesis of microvascular dysfunction. *Diabetes Care*, 1999. 22(9): p. 1549-1554.
5. Veves A, Akbari CM, Primavera J, Donaghue VM, Zacharoulis D, Chrzan JS, DeGirolami U, LoGerfo FW, Freeman, R, Endothelial dysfunction and the expression of endothelial nitric oxide synthetase in diabetic neuropathy, vascular disease, and foot ulceration. *Diabetes*, 1998. 47(3): p. 457-63.
6. Vinik AI, Erbas T, Park TS, Pierce KK, Stansberry KB, Methods for evaluation of peripheral neurovascular dysfunction. *Diabetes Technology & Therapeutics*, 2001. 3(1): p. 29-50.
7. Kilo S, Berghof M, Hilz M, Freeman R, Neural and endothelial control of the microcirculation in diabetic peripheral neuropathy. *Neurology*, 2000. 54(6): p. 1246-1252.
8. Boulton AJM, Scarpello JHB, Ward JD, Venous Oxygenation in the Diabetic Neuropathic Foot - Evidence of Arteriovenous Shunting. *Diabetologia*, 1982. 22(1): p. 6-8.
9. Edmonds ME, Roberts VC, Watkins PJ, Blood-Flow in the Diabetic Neuropathic Foot. *Diabetologia*, 1982. 22(1): p. 9-15.
10. Fagrell B, Jörneskog G, Intaglietta M, Disturbed microvascular reactivity and shunting - a major cause for diabetic complications. *Vasc Med*, 1999. 4(3): p. 125-7.

11. Jörneskog G, Brismar K, Fagrell B, Skin Capillary Circulation Severely Impaired in Toes of Patients with Iddm, with and without Late Diabetic Complications. *Diabetologia*, 1995. 38(4): p. 474-480.
12. Ward JD, Simms JM, Knight G, Boulton AJM, Sandler DA, Venous Distension in the Diabetic Neuropathic Foot (Physical Sign of Arteriovenous Shunting). *J R Soc Med*, 1983. 76(12): p. 1011-1014.
13. Fredriksson I, Larsson M, Strömberg T, Measurement depth and volume in laser Doppler flowmetry. *Microvasc Res*, 2009. 78(1): p. 4-13.
14. Kellogg DL, Zhao JL, Wu Y, Endothelial nitric oxide synthase control mechanisms in the cutaneous vasculature of humans in vivo. *Am J Physiol Heart Circ Physiol*, 2008. 295(1): p. H123-H129.
15. Minson CT, Berry LT, Joyner MJ, Nitric oxide and neurally mediated regulation of skin blood flow during local heating. *J Appl Physiol*, 2001. 91(4): p. 1619-26.
16. Gooding KM, Hannemann MM, Tooke JE, Clough GF, Shore AC, Maximum skin hyperaemia induced by local heating: Possible mechanisms. *Journal of Vascular Research*, 2006. 43(3): p. 270-277.
17. Lenasi H, Strucl M, The effect of nitric oxide synthase and cyclooxygenase inhibition on cutaneous microvascular reactivity. *Eur J Appl Physiol*, 2008. 103(6): p. 719-726.
18. Medow MS, Glover JL, Stewart JM, Nitric oxide and prostaglandin inhibition during acetylcholine-mediated cutaneous vasodilation in humans. *Microcirculation*, 2008. 15(6): p. 569-579.
19. Rossi M, Carpi A, Galetta F, Franzoni F, Santoro G, The investigation of skin blood flowmotion: a new approach to study the microcirculatory impairment in vascular diseases? *Biomed Pharmacother*, 2006. 60(8): p. 437-442.
20. Schmidt C, Adechokan S, Mouhli J, Laser-Doppler flowmetry in peripheral arterial occlusive disease. Correlations with transcutaneous oximetry. *J Mal Vasc*, 1996. 21(5): p. 294-298.

21. Tenland T, Salerud EG, Nilsson GE, berg PÅ, Spatial and temporal variations in human skin blood flow. *Int J Microcirc Clin Exp*, 1983. 2(2): p. 81-90.
22. Fredriksson I, Larsson M, Strömberg T, Model-based quantitative laser Doppler flowmetry in skin. Accepted for publication in *J Biomed Opt*, 2010.
23. Dahlén EM, Länne T, Engvall J, Lindström T, Grodzinsky E, Nyström FH, Östgren CJ, Carotid intima-media thickness and apolipoprotein B/apolipoprotein A-I ratio in middle-aged patients with Type 2 diabetes. *Diabet Med*, 2009. 26(4): p. 384-90.
24. Wijkman M, Länne T, Engvall J, Lindström T, Östgren CJ, Nyström FH, Masked nocturnal hypertension--a novel marker of risk in type 2 diabetes. *Diabetologia*, 2009. 52(7): p. 1258-64.
25. Larsson M, Strömberg T, Toward a velocity-resolved microvascular blood flow measure by decomposition of the laser Doppler spectrum. *J Biomed Opt*, 2006. 1(1): p. 014024.
26. Nilsson GE, Salerud EG, Strömberg T, Wårdell K, Laser Doppler Perfusion Monitoring and Imaging. In *Biomedical photonics handbook*, Vo-Dinh T, Editor. 2003, CRC Press: Boca Raton, FL. p. 15-1 - 15-24.
27. Vinik AI, Erbas T, Park TS, Stansberry KB, Scanelli JA, Pittenger GL, Dermal Neurovascular Dysfunction in Type 2 Diabetes. *Diabetes Care*, 2001. 24(8): p. 1468-1475.
28. Nilsson GE, Signal processor for laser Doppler tissue flowmeters. *Med Biol Eng Comput*, 1984. 22(4): p. 343-8.
29. Barnett NJ, Dougherty G, Pettinger SJ, Comparative study of two laser Doppler blood flowmeters. *J Med Eng Technol*, 1990. 14(6): p. 243-9.
30. Petoukhova AL, Steenbergen W, Morales F, Graaff R, de Jong ED, Elstrodt JM, de Mul FFM, Rakhorst G, Instrument-independent flux units for laser Doppler perfusion monitoring assessed in a multi-device study on the renal cortex. *Microvasc Res*, 2003. 66(2): p. 83-90.

Table 1. Characteristics of diabetes patients (DM) and non-diabetes controls (ND).

	ND (N = 23)	DM (N = 23)
Gender (M/F)	8/15	14/9
Age [years]	62 ± 6	60 ± 3
Diabetes duration [years]	N/A	7 ± 6
Height (Male) [cm]	178 ± 7	176 ± 7
Height (Female) [cm]	166 ± 7	164 ± 5
Weight (Male) [kg]	84 ± 16	91 ± 8
Weight (Female) [kg]	67 ± 10	78 ± 9 *
BMI [kg/m <sup>2</sup> ]	25 ± 3	29 ± 3 ‡
Glucose [mmol/l]	6.0 ± 0.8 §	8.1 ± 2.1 ‡§
Triglyceride [mmol/l]	1.3 ± 0.8	1.9 ± 1.4 *¶
Cholesterol [mmol/l]	5.8 ± 1.0	4.2 ± 0.6 ‡¶
LDL-C [mmol/l]	3.5 ± 0.8 §	2.4 ± 0.6 ‡#
HDL-C [mmol/l]	1.7 ± 0.6	1.1 ± 0.3 ‡¶
HbA <sub>1c</sub> [%]	N/A	6.0 ± 0.9 §
Systolic blood pressure [mmHg]	120 ± 12	122 ± 10
Diastolic blood pressure [mmHg]	74 ± 8	72 ± 7
Pulse wave velocity [m/s]	8.9 ± 1.7	11.2 ± 2.0 ‡¶
Left ventricle mass (Male) [g]	106 ± 11	127 ± 34
Left ventricle mass (Female) [g]	106 ± 29	93 ± 36 §
Carotid IMT [mm]	0.67 ± 0.12	0.75 ± 0.13 *
TBI [-]	0.89 ± 0.16 ¶	0.93 ± 0.12

Statistical comparisons of DM vs. ND, \* p < 0.05, ‡ p < 0.001.

§ One value missing; || Two values missing; ¶ Three values missing; # Five values missing.

Table 2. Conventional [c; a.u.] and quantitative [q; g RBC / 100 g tissue × mm/s] LDF perfusion estimates (mean ± s.d.) at baseline (B; median over first 5 minutes) and at the plateau (Pl; median over last 5 minutes). cLDF was measured using 0.25 and 1.2 mm fiber separations. qLDF was measured in different velocity regions.

LDF	Time	Fiber sep/velocity	ND (N = 23)	DM (N = 23)
c	B	0.25 mm sep.	46 ± 12	46 ± 15 ¶¶
c	B	1.2 mm sep.	242 ± 84	260 ± 55 ¶¶
q	B	v < 1 mm/s	0.019 ± 0.012	0.016 ± 0.005 ¶¶
q	B	1 < v < 10 mm/s	0.016 ± 0.009	0.019 ± 0.009 ¶¶
q	B	v > 10 mm/s	0.026 ± 0.012	0.032 ± 0.015 ¶¶
q	B	all v	0.061 ± 0.025	0.067 ± 0.019 ¶¶
c	Pl	0.25 mm sep.	485 ± 215	335 ± 113 †
c	Pl	1.2 mm sep.	1842 ± 562	1491 ± 396 *
q	Pl	v < 1 mm/s	0.017 ± 0.019	0.021 ± 0.018
q	Pl	1 < v < 10 mm/s	0.20 ± 0.08	0.16 ± 0.06
q	Pl	v > 10 mm/s	0.77 ± 0.42	0.53 ± 0.26 *
q	Pl	all v	0.98 ± 0.44	0.71 ± 0.28 *

¶¶ The baseline measurements were excluded for three DM patients due to too short acclimatization.

Statistical comparisons DM vs. ND, \* p < 0.05; † p < 0.01.

Table 3. Conventional [c; a.u.] and quantitative [q; g RBC / 100 g tissue] LDF mean concentration of moving blood cells (mean  $\pm$  s.d.) at baseline (B; median over first 5 minutes), and at the plateau (Pl; median over last 5 minutes). cLDF was measured using 0.25 and 1.2 mm fiber separations. qLDF was measured in different velocity regions.

LDF	Time	Fiber sep/velocity	ND ( $N = 23$ )	DM ( $N = 23$ )
c	B	0.25 mm sep.	0.61 $\pm$ 0.18	0.57 $\pm$ 0.16 ¶
c	B	1.2 mm sep.	0.86 $\pm$ 0.14	0.83 $\pm$ 0.084 ¶
q	B	$v < 1$ mm/s	0.085 $\pm$ 0.064	0.069 $\pm$ 0.027 ¶
q	B	$1 < v < 10$ mm/s	0.0064 $\pm$ 0.0034	0.0075 $\pm$ 0.0034 ¶
q	B	$v > 10$ mm/s	0.00076 $\pm$ 0.00032	0.00091 $\pm$ 0.00033 ¶
q	B	<i>all v</i>	0.092 $\pm$ 0.065	0.077 $\pm$ 0.027 ¶
c	Pl	0.25 mm sep.	0.78 $\pm$ 0.12	0.74 $\pm$ 0.14
c	Pl	1.2 mm sep.	0.96 $\pm$ 0.05	0.96 $\pm$ 0.046
q	Pl	$v < 1$ mm/s	0.039 $\pm$ 0.043	0.061 $\pm$ 0.090
q	Pl	$1 < v < 10$ mm/s	0.056 $\pm$ 0.022	0.048 $\pm$ 0.017
q	Pl	$v > 10$ mm/s	0.018 $\pm$ 0.0069	0.013 $\pm$ 0.0052 *
q	Pl	<i>all v</i>	0.11 $\pm$ 0.051	0.12 $\pm$ 0.083

¶ The baseline measurements were excluded for three DM patients due to too short acclimatization.

Statistical comparisons DM vs. ND, \*  $p < 0.05$ .

Table 4. Conventional [c; a.u.] and quantitative [q; mm/s] LDF mean velocity estimates (mean  $\pm$  s.d.) at baseline (B; median over first 5 minutes), and at the plateau (Pl; median over last 5 minutes). cLDF was measured using 0.25 and 1.2 mm fiber separations. qLDF was measured in different velocity regions.

LDF	Time	Fiber sep/velocity	ND ( $N = 23$ )	DM ( $N = 23$ )
c	B	0.25 mm sep.	$78 \pm 20$	$84 \pm 26$ ¶
c	B	1.2 mm sep.	$288 \pm 110$	$313 \pm 66$ ¶
q	B	<i>all v</i>	$0.83 \pm 0.40$	$0.94 \pm 0.37$ ¶
c	Pl	0.25 mm sep.	$627 \pm 317$	$461 \pm 165$ *
c	Pl	1.2 mm sep.	$1925 \pm 629$	$1558 \pm 438$ *
q	Pl	<i>all v</i>	$9.7 \pm 4.7$	$7.3 \pm 3.9$ *

¶ The baseline measurements were excluded for three DM patients due to too short acclimatization.

Statistical comparisons DM vs. ND, \*  $p < 0.05$ .

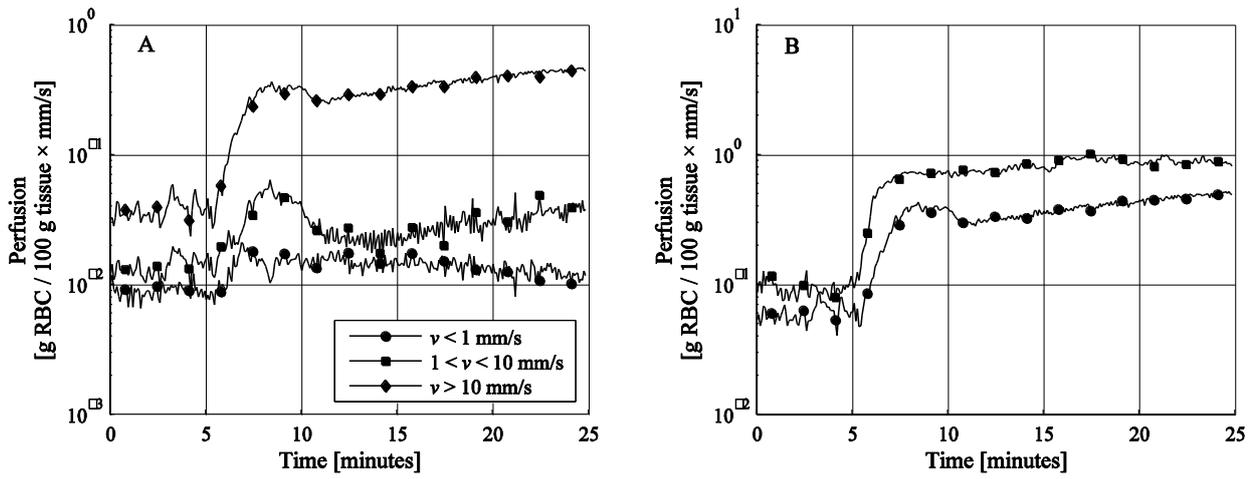


Figure 1. Typical response of the perfusion in the three different velocity regions (A) and two typical responses of the total perfusion (B).

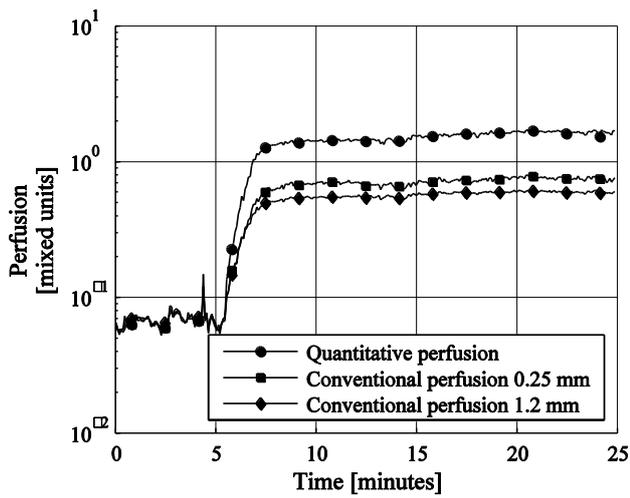


Figure 2. Differences in response to heat stimuli between the qLDF perfusion estimate and the cLDF perfusion estimates at the two fiber separations. The cLDF estimates are normalized to equal the average baseline level of the qLDF perfusion estimate.

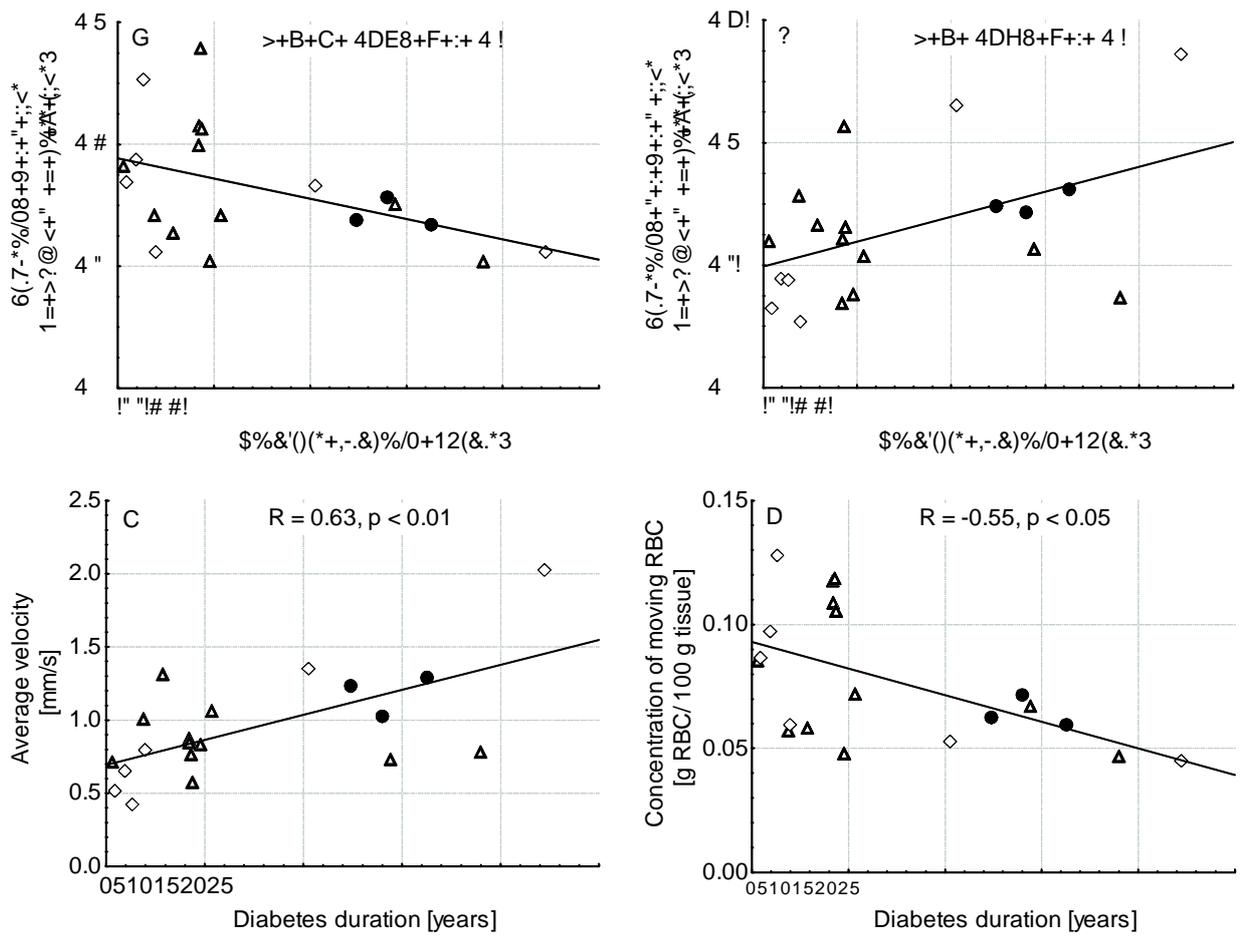


Figure 3. Relation between diabetes duration and quantitative LDF perfusion in low- (A) and mid-velocity (B) region, average velocity (C) and the concentration of moving red blood cells (D) at baseline. Diabetes treatments were insulin (filled circle), oral hypoglycemic agents (triangle) and diet and exercise (rhomb), respectively.

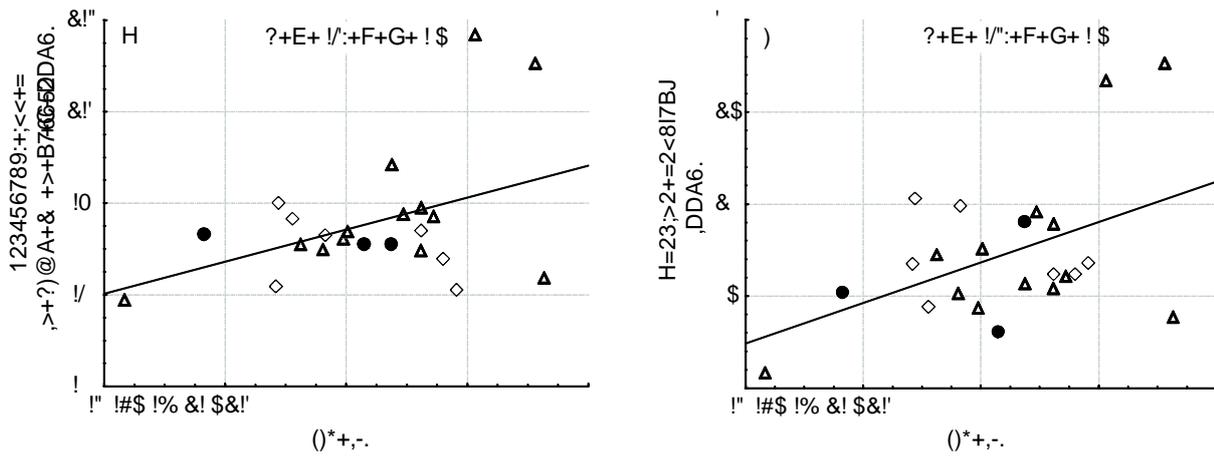


Figure 4. Relation between TBI (toe-to-brachial systolic pressure ratio) and quantitative LDF perfusion for all velocities (A) and the average velocity of moving red blood cells (B) after heat. Diabetes treatments were insulin (filled circle), oral hypoglycemic agents (open triangle) and diet and exercise (open rhomb), respectively.