Influence of Repetitive Finger Puncturing on Skin Perfusion and Capillary Blood Analysis in Patients with Diabetes Mellitus

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Background: Frequent puncturing of fingers to check blood glucose in patients with type 1 diabetes might alter skin perfusion and, hence, influence the representativeness of the blood sample. We investigated the influence of repetitive puncturing on skin microcirculatory perfusion using laser Doppler fluxmetry and on the preanalytical phase of capillary blood analysis for small molecules (glucose) and large particles (cholesterol).

Methods: In 49 patients with long-standing (mean, 21 years) type 1 diabetes, with a mean puncture frequency of three times daily for a mean duration of 13 years, laser Doppler skin perfusion was measured in a finger at a frequently punctured site and compared with a similar site of another finger of the same hand, which was never punctured. In the supine position with the hand level with the heart, resting flux (RF), peak flux (PF), and the microcirculatory reserve capacity (MRC; PF−RF) were assessed. Subsequently, blood samples for capillary whole blood glucose and cholesterol analyses were taken from the same sites.

Results: No significant differences were found between the puncture and control sites in mean RF (2.3 vs 2.0 V; P = 0.14, paired-samples t-test), PF (3.3 vs 3.1 V; P = 0.24), MRC (1.0 vs 1.0 V; P = 0.65), glucose (10.2 vs 10.2 mmol/L; P = 0.69), or cholesterol (5.1 vs 5.2 mmol/L; P = 0.26). Power calculation for a RF of 2.0 V and the SD and n of this study indicate a power (β) of 80% to detect a 25% change in RF at P < 0.05.

Conclusions: Repetitive finger puncturing in diabetics appears not to injure local skin microcirculatory perfusion nor to influence results of capillary blood analysis for glucose and cholesterol.

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Patients with type 1 diabetes usually check their blood glucose by means of microblood sampling from one or more finger tips. Intensive therapy in patients with type 1 diabetes delays the onset and slows the progression of clinically important organ failure (1). For this purpose, frequent puncturing for glucose analysis is required. The technique for capillary blood glucose analysis is minimally invasive and causes minimal lesions. Often, however, the same place is used several times a day. This practice may hypothetically influence local skin microcirculation and therefore influence the reliability of the capillary blood tests.

After skin injury, wound healing consists of three phases: the lag phase, representing the acute inflammatory response; the proliferative phase, which is characterized by synthesis and deposition of new connective tissue matrix; and the remodeling phase, which is the maturation of the newly deposited tissue. After a short period of vasoconstriction to prevent excessive bleeding, all three phases are accompanied by an increased tissue perfusion (2, 3). Clinical evidence has shown that skin trauma produces a local hyperemic response as a result of a local (sterile) inflammatory reaction following a 10-min vasoconstriction (4).

In general, the extent and duration of the skin response to a local trauma are influenced by the severity and location of the trauma. Previous studies showed that the hyperemic response to injury is fast and the time to peak after injury varies between 15 s (4) and 15 min (5, 6). The duration of hyperemia varies with the size and depth of the injury. The response to a single needle puncture varies between 15 min (4) and 50 h (7, 8), whereas the hyperemia may exceed more than 1 year in hypertropic scar formation (9). The duration of the hyperemic response...
after microblood sampling for microglucose analysis has never been studied. The frequent puncturing in diabetics could theoretically lead to a local permanent or semi-permanent increase of the basal perfusion.

The reliability of laboratory investigations is dependent on the variation in the preanalytical and analytical phases. The variation in the analytical phase is declining because of technical improvements; therefore, the preanalytical variation is gradually becoming the key factor influencing the reliability of a test. The preanalytical phase is influenced by biological variation, choice of specimen, specimen collection, and transport. The biological variation (e.g., diet, obesity, smoking, exercise, alcohol intake, metabolic state, illness, and diurnal variation) is most important and averages, for example, 60% of the total intradividual variation of cholesterol determination (10, 11). The biological variation in cholesterol analysis is influenced by changes in posture, sympathetic nervous activity, blood volume, and hemocoagulation (12, 13). Likewise, glucose analysis is related to hematocrit, sample site (arterial-venous glucose gradients), tissue perfusion, and temperature (14, 15).

Hypothetically, repetitive puncturing would produce higher skin perfusion. This putative effect on skin microcirculation may have important repercussions on the widespread routine assessment of the serum concentrations of small, water-soluble molecules (glucose) or larger, non-water-soluble particles (cholesterol) derived from capillary blood samples. Therefore, we investigated the influence of repetitive finger puncturing on skin microcirculatory perfusion, using laser Doppler fluxmetry, and on capillary whole blood glucose and total cholesterol analysis in patients with longstanding type 1 diabetes.

**Patients and Methods**

**Patients**

A total of 49 patients (22 men and 27 women) were selected from the outpatient Department of Internal Medicine of the Academic Medical Center if they had longstanding type 1 diabetes and punctured their fingers for glycemia checking for at least 3 years. Subjects were enrolled after giving written informed consent. The mean age was 43 years (range, 24–77 years). The mean diabetes duration was 21 years (range, 7–39 years). The “finger puncture frequency” was defined as the number of finger punctures per week divided by the total number of fingers used for puncturing. The severity of long-term complications of diabetes mellitus was estimated from medical history scoring the absence (0 points) or presence (1 point) of retinopathy (as assessed by an ophthalmologist), nephropathy (microalbuminuria >30 mg/24 h) (16), and the need for use of angiotensin-convertase enzyme inhibitors irrespective of microalbuminuria, macroangiopathy (myocardial infarction, cerebrovascular accident, or feet ulcers), and neuropathy (sensory disturbances of the feet). Thus, the total score ranged from 0 to 5 points. The extent of macroscopic skin changes caused by puncturing was classified in two groups: barely and clearly visible.

**Skin Perfusion**

Local skin microcirculatory perfusion was assessed by laser Doppler fluxmetry (Periflux 4001®; PF 408 standard probe; Perimed) (17), a simple, noninvasive technique to assess total cutaneous blood flow (18–20). In short, laser light with a wavelength of 780 nm is conducted through optical fibers to the skin where it penetrates the skin to a depth of 1–1.5 mm and is partly reflected. When backscattered by moving objects (principally erythrocytes), this light undergoes a frequency shift, which is proportional to the velocity and number of moving objects (flux), and is expressed in volts [laser Doppler flux (LDF)]. Laser Doppler measures perfusion not only in the capillaries but also in the subpapillary venular and arteriolar plexus and arteriovenous shunts (17).

Measurements were performed on the distal phalanx of the finger at the site most frequently used for microblood sampling and compared with a similar site of another finger of the same hand never used for this purpose. Unheated probes were attached to the fingertips with the probe holder (Perimed) and double-sided adhesive tape. The instrument’s time constant was set at 3 s. Measurements were performed in the supine position with the hand level with the heart in a temperature-controlled environment (22–24 °C) after an acclimatization period of 15 min. Recordings were sampled on-line and analyzed off-line by means of a data acquisition system (AcqKnowledge III and MP 100WSW; Biopac System).

Resting flux (RF) values (in volts) were obtained by averaging the recording during 5 min. Subsequently, peak flux (PF; in volts) was assessed during reactive hyperemia following a 3-min arterial occlusion induced by inflating a cuff around the arm to 200 mmHg. Because it is known that the spatial variation of laser Doppler measurements is considerable (CV, 21–51%) (21, 22), the increase in LDF as a measure of microcirculatory reserve capacity (MRC) was calculated. The MRC was defined as PF minus RF. In addition, we performed an evaluation of the LDF differences between the third and fourth finger of the same hand in a control group of 15 healthy volunteers. Biological zero as obtained during arterial occlusion was subtracted from all flux values (23).

**Capillary Blood Analysis**

Capillary whole blood cholesterol (Lipotrend C®; Boehringer) (24) and glucose tests (Glucometer Elite®; Bayer) (25) were performed at the same measurement sites of both fingers after the LDF returned to baseline (~5 min). Glucose testing was performed in only the latter 32 of 49

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4 Nonstandard abbreviations: LDF, laser Doppler flux; RF, resting flux; PF, peak flux; MRC, microcirculatory reserve capacity; and CI, confidence interval.
patients because the test was not available from the beginning of this study. A 1.4-gauge needle (Brand Safety Flow Lancets, Microtainer®; Becton Dickinson) was used for puncturing. The first drop of blood was discarded. Blood was drawn into the glucose analysis strip and into a heparin-coated capillary tube (30 μL), after which it was applied to the test zone and inserted into the instrument for cholesterol analysis. The analyses were performed according to the manufacturer’s instructions. Both glucose and cholesterol concentrations were assessed to detect whether the effect of frequent puncturing on microblood sampling would be different between small water-soluble molecules (glucose) and larger non-water-soluble particles (cholesterol). This last factor was also introduced because it is to be expected that capillary blood analyses of a wide range of blood substances will be performed increasingly in clinical chemistry.

**STATISTICAL METHODS**

The results are expressed as means with SD after testing for skewness. Differences in laser Doppler parameters and capillary blood chemistry between the puncture and control sites were analyzed using the Student t-test for paired samples. Mean differences (d; control vs puncture site) with SD and 95% confidence intervals (95% CIs) are presented for all values. Differences between sites across the range of values were analyzed visually by means of a difference plot (26).

A possible correlation between finger puncture frequency and microcirculatory changes (RF, PF, and MRC) was investigated by the Pearson product–moment correlation coefficient. We assumed a positive correlation between the finger puncture frequency and RF at the puncture site and a negative correlation between the finger puncture frequency and MRC at the puncture site because repetitive puncturing should produce a semi-permanent reactive hyperemia. Because no adequate values of the technique used were available before the study, power analyses for the RF values were performed retrospectively to calculate the power that can be reached regarding the observed differences.

**Results**

Patients had diabetes mellitus for a mean period of 21 years (range, 7–39 years), and performed capillary blood sampling for a mean period of 13 years (range, 3–33 years), with a mean sampling frequency of 17 times a week (range, 1–50). One to eight fingers of both hands were used for puncturing. The mean finger puncture frequency was 5 times a week (range, 1–18 times a week). The visibility of the puncture sites varied from invisible to clearly present.

**SKIN PERFUSION**

In control subjects, the CV of differences between the third and fourth finger was 54% for rest LDF [(SD differences/control site) × 100%; 1.3/2.4 × 100%], 39% (1.5/3.8 × 100%) for peak LDF, and 36% (0.5/1.4 × 100%) for MRC.

The LDF values at rest and during reactive hyperemia are shown in Table 1. No significant differences in laser Doppler perfusion parameters were found between the puncture and control sites. These flux differences were unequally distributed across the range. The differences tended to increase with higher flux values (Fig. 1), in agreement with the greater spatial and temporal variation that is known to exist at higher flux values (22). The RF, PF, and MRC did not differ significantly between the sites with barely and clearly visible puncture marks. Power analysis revealed that in these 49 patients and with an SD of the mean differences of 1.2 V, a 25% flux difference (0.5 V) between the control (2.0 V) and puncture site (2.5 V) can be observed with \( P = 0.05 \) and a power (\( \beta \)) of 80%.

**CAPILLARY BLOOD ANALYSIS**

The mean (± SD) capillary blood glucose in the puncture site (10.1 ± 4.7 mmol/L) was not different (\( P = 0.69 \)) from the control site (10.2 ± 4.4 mmol/L); mean (± SD) difference, 0.09 ± 1.32 mmol/L; 95% CI, −0.19 to 0.28 mmol/L. The same was true for the capillary cholesterol (5.1 ± 1.1 mmol/L for the puncture site vs 5.2 ± 1.0 mmol/L for the control site; mean difference, 0.07 ± 0.40 mmol/L; 95% CI, −0.05 to 0.18 mmol/L; \( P = 0.26 \)).

The distribution was equal across the range of concentrations of glucose and cholesterol (Fig. 2), illustrating that neither an actual change in the control of the diabetes mellitus nor the presence of hypercholesterolemia produced a greater difference.

**CORRELATIONS**

A poor correlation was found between finger puncture frequency and microcirculatory parameters at the puncture and control sites, indicating that frequent puncturing did not influence skin perfusion (Table 2). Small but significant inverse correlations were seen between both duration of type 1 diabetes and severity of long-term complications and RF at the puncture site (Table 2), whereas only a poor correlation was observed at the

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Punctured site (SD)</th>
<th>Control site (SD)</th>
<th>d ± SD</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF, V</td>
<td>2.29 (1.26)</td>
<td>2.01 (1.38)</td>
<td>−0.28 ± 1.18</td>
<td>−0.61 to 0.16</td>
<td>0.1</td>
</tr>
<tr>
<td>PF, V</td>
<td>3.27 (1.14)</td>
<td>3.05 (1.59)</td>
<td>−0.22 ± 1.34</td>
<td>−0.62 to 0.06</td>
<td>0.24</td>
</tr>
<tr>
<td>MRC, V</td>
<td>0.98 (0.72)</td>
<td>1.04 (0.79)</td>
<td>0.06 ± 0.88</td>
<td>−0.20 to 0.31</td>
<td>0.65</td>
</tr>
</tbody>
</table>

* Mean values are shown, including mean differences between puncture and control values (d) with SD, 95% CI, and \( P \) for the paired Student t-test; \( n = 49 \).
control site. The PF showed the same tendencies, but the correlations were not statistically significant ($P = 0.09$ and 0.10).

**Discussion**

This study presents the first evidence that repetitive finger puncturing in patients with long-standing insulin-dependent diabetes mellitus does not influence microcirculatory skin perfusion. Likewise, the capillary blood glucose and cholesterol concentrations are not influenced by repetitive puncturing, and thus preanalytical variation attributable to altered circulation does not need to be considered.

**SKIN PERFUSION**

In contrast to our hypothesis, we did not observe a difference in skin perfusion between the puncture and control fingers. Both methodological and pathophysiological factors may contribute to this observation.

A methodological reason for this could be that the effect on skin microcirculation was too small to measure with laser Doppler. However, the rather narrow 95% CIs of the mean differences in LDF values and the power analyses indicate that the sample size was adequate. Measurement variability of the laser Doppler is mainly attributable to the large physiological variation in perfusion rather than the technical variation of the technique used (21, 22, 27, 28). The physiological variation among persons and different anatomic sites is larger than the within-person variation at identical anatomical sites of the same limb (22, 28–30), as was used in this study. The use of postocclusive hyperemic tests further improves the reproducibility of the measurement (31). Furthermore, no
differences were observed between the various fingers of the same and between left and right hands (31). In previous studies, we demonstrated a difference in laser Doppler perfusion between the stages of peripheral vascular disease (32, 33) and reflex sympathetic dystrophy (34), whereas many others have demonstrated differences in skin perfusion induced by skin injury, using laser Doppler fluxmetry (4, 7–9, 35). Therefore, the laser Doppler technique is applicable in long-term studies on factors affecting microcirculatory flow (27) and should be able to detect important changes in microcirculation if present.

Pathophysiological factors for this lack of difference may include the following: (a) The injury caused by puncturing for microblood sampling may not be big enough to induce longstanding hyperemia. (b) The increase of blood flow that accompanies wound healing (vasodilatation and neoangiogenesis) might not be detected by laser Doppler because scar formation increases the nonperfused part of the 1.5 mm³ of skin measured by laser Doppler, thereby reducing the backscattered laser Doppler signal (17). However, there was no significant difference in perfusion between the fingers with barely and clearly visible macroscopic changes. (c) The local microcirculatory anatomy, which varies throughout the body, may contribute to the observed absence of a difference between the puncture and control site. Experimental studies have shown that the hyperemic response in skin areas with a primarily thermoregulatory (arteriovenous) perfusion, such as the volar sides of the fingers, appears not as pronounced as in other regions of the skin with primarily nutritive perfusion (8, 36–38). (d) Diabetic microangiopathy may contribute to the results of this study. The hyperemic response after, for example, needle injury, heating, or arterial occlusion is known to be reduced in patients with type 1 diabetes (5, 7). The cause of the inability of the diabetic skin to respond normally to injury is complex and is part of the microcirculatory changes (increased RF with impaired reactive hyperemia) that take place in patients with diabetes mellitus and are called functional diabetic microangiopathy (39). The influence of diabetic microangiopathy on the reduction of hyperemia after repetitive puncturing is supported by the inverse correlation we found between skin perfusion in the puncture site and duration of diabetes mellitus and severity of long-term complications. However, this was not observed at the control site, which argues against such an explanation.

Table 2. Correlations* between laser Doppler fluxmetry and finger puncture frequency, duration of diabetes mellitus, and severity of long-term complications.

<table>
<thead>
<tr>
<th>FPF</th>
<th>Control site (n = 49)</th>
<th>Puncture site (n = 49)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RF</td>
<td>Duration</td>
</tr>
<tr>
<td></td>
<td>-0.22 0.14</td>
<td>-0.11 0.47</td>
</tr>
<tr>
<td></td>
<td>-0.16 0.26</td>
<td>-0.03 0.84</td>
</tr>
<tr>
<td></td>
<td>0.05 0.75</td>
<td>0.13 0.39</td>
</tr>
<tr>
<td></td>
<td>MRC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.19 0.20</td>
<td>-0.31 0.03</td>
</tr>
<tr>
<td></td>
<td>-0.16 0.28</td>
<td>-0.24 0.10</td>
</tr>
<tr>
<td></td>
<td>-0.02 0.88</td>
<td>0.17 0.24</td>
</tr>
</tbody>
</table>

*Pearson’s product-moment (r) and Spearman’s rank-order (p) correlation coefficient with probability (P) and between-finger puncture frequency, duration of diabetic mellitus, severity of long-term complications, and RF, PF, and MRC at the control and puncture sites.

FPF, between-finger puncture frequency.

Fig. 2. Scatter plots of the differences (control – puncture site; y-axis) in capillary whole blood glucose (A) and capillary whole blood cholesterol (B) vs the mean (x-axis).

The mean difference (solid line) and the 95% CI (± 1.96 SD; dotted lines) of the differences are presented.
In agreement with the findings in the microcirculation, capillary blood glucose and cholesterol analysis were not influenced by repetitive puncturing. Methodological factors (too small an effect or sample size) may have caused this finding. Nevertheless, if repetitive puncturing does have an effect on capillary blood analysis, the influence appears too small to be clinically significant.

CONCLUSIONS
The results of this study show that, within the limitations of the used assay, repetitive finger puncturing does not influence skin microcirculation and, hence, the preanalytical phase of capillary blood analysis. Apparently, frequent finger puncturing does not induce a trauma that induces a lasting, local hyperemic response. Even if puncturing should induce an acute hyperemic response, this appears to have no lasting effect or may be surpassed by the reduced hyperemic response caused by diabetic microangiopathy. In conclusion, repetitive puncturing has no clinical consequences for capillary whole blood glucose and cholesterol analysis.

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References
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