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# Does combined kidney and pancreas transplantation reverse functional diabetic microangiopathy?

**G. Jörneskog[1], J. Östergren[1], G. Tydén[2], J. Bolinder[3], and B. Fagrell[1]**

[1] Department of Internal Medicine, Karolinska Institute, Karolinska Hospital, Box 60500, S-10401 Stockholm, Sweden
[2] Department of Transplantation Surgery and [3] Department of Internal Medicine, Karolinska Institute, Huddinge Hospital, S-14186 Huddinge, Sweden

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**Abstract.** Using videophotometric capillaroscopy and laser Doppler fluxmetry, we have investigated skin microvascular reactivity in the fingers of 14 diabetic patients with severe, late complications 20 months after combined kidney and pancreas transplantation. The results were compared with those obtained in 20 diabetic patients awaiting pancreas transplantation and in 19 healthy subjects. The capillary blood cell velocity at rest ($P < 0.01$) and during postocclusive reactive hyperemia ($P < 0.05$) was significantly lower in both patient groups than in the healthy controls. However, the time to peak capillary blood cell velocity during hyperemia was normal in the post-transplantation group (NS) but significantly prolonged in the pretransplantation group ($P < 0.01$). The ability to decrease flow during venous stasis – the so called venoarte-riolar reflex – was strongly impaired in the pretransplantation group ($P < 0.001$) but less so in the post-transplantation group ($P < 0.05$) as compared to healthy controls. It may be concluded that diabetic patients, after combined kidney and pancreas transplantation, show a tendency towards better microvascular reactivity than those awaiting transplantation.

**Key words:** Pancreas transplantation, microangiopathy – Microangiopathy, pancreas transplantation

Following successful pancreas transplantation, the diabetic patient becomes insulin-independent and the various metabolic parameters are normalized or near normalized [32]. This undoubtedly improves the patient's quality of life. However, instead of insulin, the patient must most likely start on lifelong immunosuppressive therapy. The main aim of the transplantation is to reverse or halt the secondary complications of diabetes, but whether this can be achieved by a normalization of the metabolic abnormalities is still subject to debate. In two recent studies, no effect of pancreas transplantation could be found on diabetic retinopathy or neuropathy, respectively [24, 27]. However, the diabetic patients included in these studies all had end-stage nephropathy, and it is likely that these patients had secondary complications that were too far advanced to be halted. In another study of non-uremic diabetic recipients of pancreatic grafts, a beneficial effect was noted on the diabetic neuropathy [33]. The lack of specific techniques has, until recently, made it difficult to objectively evaluate the effect of diabetic microangiopathy on the reactivity of human microcirculation. However, by using videophotometric capillaroscopy and laser Doppler fluxmetry, the dynamics of skin microcirculation can be quantified [5, 8–10, 29], and we have previously demonstrated an impaired regulatory capacity of skin microcirculation in fingers of type 1 diabetics [11, 22, 23, 28, 30, 31]. The purpose of the present study was to investigate the effect of an improved metabolism on skin microvascular reactivity in diabetic patients who had undergone successful combined kidney and pancreas transplantation.

*Offprint requests to:* G. Jörneskog

## Subjects and methods

### *Subjects*

Group 1 consisted of 20 (9 male, 11 female) type 1 diabetics (insulin-dependent) awaiting pancreas transplantation or combined kidney and pancreas transplantation (CKPT) because of severe, late diabetic complications. Their mean age was 35 years and the mean duration of diabetes 23 years.

Group 2 comprised 14 (9 male, 5 female) type 1 diabetics (insulin-dependent) who, 20 months (median; range 9–51 months) earlier, had undergone CKPT because of severe, late diabetic complications. Age and duration of diabetes were both similar to those of patients in group 1. All had functioning grafts and normal blood glucose levels without exogenous insulin at investigation. The results of intravenous glucose tolerance tests were normal or near-normal ($k = 1.1\% \pm 0.5\%$/min).

Group 3 included 19 healthy subjects (12 male, 7 female) of the same age as patients in groups 1 and 2.

Subject characteristics are summarized in Table 1.

### *Methods*

The skin microcirculation of the nailfold of the third or fourth finger of the left hand was studied using videophotometric capillaroscopy and laser Doppler fluxmetry. All subjects were acclimatized for 30 min at 23° ± 1°C before measurements were taken. Smokers were asked to refrain from smoking 2 h prior to the investigation [20]. Subjects were seated with the left arm supported at heart level on a table. A miniature cuff was applied to the base of investigated finger so that arterial and venous occlusions could be performed [11]. The

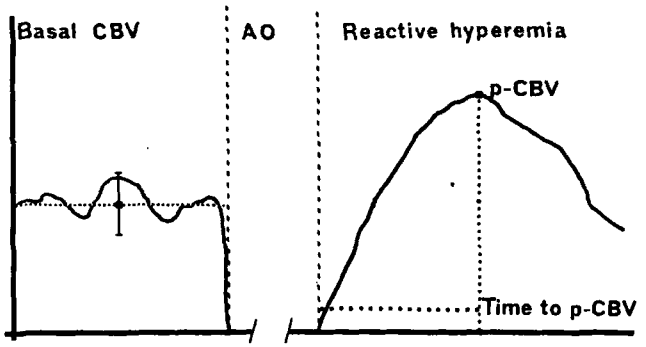


**Fig. 1.** Schematic illustration of the variables used for evaluating the reactive hyperemia response in skin capillaries. *CBV*, Capillary blood cell velocity (mm/s); *AO*, arterial occlusion; *p-CBV*, peak CBV during reactive hyperemia

skin temperature in the observed area was continuously recorded with an electronic thermistor (Exacon, Copenhagen, Denmark) placed immediately proximal to the nailfold under study.

*Videophotometric capillaroscopy.* Nailfold capillaries of a finger were visualized on a TV monitor by a Zeiss epimicroscope on which a silicon diode video camera was mounted. The image was stored on videotape for subsequent analysis. During playback of the tape, capillary blood cell velocity (CBV) was determined using a new, computerized, videophotometric, crosscorrelation technique (IM-Capiflow, Stockholm, Sweden) [8, 12]. The CBV was measured in a suitable capillary with good contrast and visible signals. This has been shown to be relevant for studying skin microvascular reactivity [20, 25]. The following variables were determined (Fig. 1):

1. Resting CBV (rCBV; mm/s)
2. Peak CBV (pCBV) following release of a 1-min arterial occlusion at the base of the finger with a cuff pressure of 200 mm Hg (mm/s)
3. Time to peak CBV (tpCBV) during reactive hyperemia (s)

*Laser Doppler fluxmetry.* To evaluate the ability to decrease skin microvascular flow during venous stasis – the so-called venoarteriolar reflex [14] – a laser Doppler fluxmeter (Periflux Model Pf2, Perimed, Stockholm, Sweden) was used [19]. The definition of flux is: velocity x number of blood cells within the measured volume. A band width of 4 kHz, a 10–100-fold gain, and a chart recording speed of 2 mm/s were employed. The laser Doppler probe was placed within the area immediately adjacent to the microscope field of view, and the percentage decrease of the laser Doppler signal (AU) during venous occlusion (voLD) was measured. Venous stasis was performed by a cuff pressure of 50 mm Hg for 30 s at the base of the finger.

*Brachial blood pressure (BP).* The BP was measured (mm Hg) in the left arm with a sphygmomanometer and with the patient in the sitting position.

*Digital systolic blood pressure (dSBP).* The dSBP was assessed (mm Hg) by recording the cuff pressure at which laser Doppler flux (AU) was seen to return when the cuff at the base of the finger was slowly deflated from suprasystolic values.

*Blood tests.* Venous blood was taken for determination of hemoglobin (Hb), hematocrit (Hct), glycosylated hemoglobin ($HbA1_c$), and serum creatinine (SCr).

### *Statistical analysis*

The results are presented as the median and range or mean ± 1 SD. The nonparametric Mann-Whitney U-test was used to determine statistically significant differences between the groups. A $P$ level of 0.05 or less was considered significant.

The study was approved by the ethics committee of Karolinska Hospital.

## Results

### *Laboratory data* (Table 2)

Diabetic control, as assessed by $HbA1_c$, was normal in groups 2 and 3, while group 1 was hyperglycemic ($P < 0.01$). As compared to group 3, kidney function was impaired in both patient groups, but most severely in group 1 ($P < 0.001$). Groups 1 ($P < 0.01$) and 2 ($P < 0.05$) showed lower levels of Hb than group 3.

### *Blood pressure* (Table 3)

The arm and finger blood pressure was elevated in both groups 1 and 2 ($P < 0.001$) as compared to that in group 3.

### *Skin microcirculation* (Table 4)

Both groups 1 and 2 showed significantly lower rCBV ($P < 0.01$) and pCBV ($P < 0.05$) than group 3. No difference was seen between the two patient groups. The tpCBV was significantly prolonged in group 1 as compared to group 2 ($P < 0.05$) and group 3 ($P < 0.01$), but no difference was seen between groups 2 and 3. The ability to decrease flow during venous stasis (vo LD) was significantly impaired in group 1 as compared to group 2 ($P < 0.05$) and group 3 ($P < 0.001$), and between groups 2

**Table 1.** Subject characteristics. CKPT, Combined kidney and pancreas transplantation

| | Group 1 Before CKPT ($n = 20$) | Group 2 After CKPT ($n = 14$) | Group 3 Healthy ($n = 19$) |
|---|---|---|---|
| Mean age (years) (SD; range) | 35 (5; 29–44) | 36 (5; 28–44) | 35 (6; 28–45) |
| Mean duration of diabetes (years) (SD; range) | 23 (6; 13–31) | 25 (5; 17–34) | 0 |
| Patients on pre-transplantation dialysis > 1 month | 4 | 2 | 0 |
| Patients treated with: | | | |
| Immunosuppressive agents | 0 | 14 | 0 |
| Anticoagulants | 0 | 14 | 0 |
| Beta blockers | 5 | 10 | 0 |
| Diuretics | 18 | 7 | 0 |
| Insulin | 20 | 0 | 0 |

**Table 2.** Laboratory data. CKPT, Combined kidney and pancreas transplantation; Hb, hemoglobin; Hct, hematocrit; $HbA1_c$, glycosylated hemoglobin; SCr, serum creatinine. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (differences as compared to group 3)

| | Normal range | Group 1 Before CKPT ($n = 20$) | Group 2 After CKPT ($n = 14$) | Group 3 Healthy ($n = 19$) |
|---|---|---|---|---|
| Hb | 120–150 g $l^{-1}$ | 111 ± 24** | 119 ± 25* | 135 ± 10 |
| Hct | 36%–45% | – | 37 ± 7* | 40 ± 4 |
| $HbA1_c$ | 4.4%–6.2% | 9.6 ± 1.8** | 4.7 ± 0.7 | 5.2 ± 0.4 |
| SCr | 0–120 μmol $l^{-1}$ | 367 ± 260*** | 193 ± 123** | 81 ± 7 |

**Table 3.** Blood pressure measurements. CKPT, Combined kidney and pancreas transplantation. * $P < 0.01$; ** $P < 0.001$ (differences as compared to group 3)

| | Group 1 Before CKPT ($n = 20$) | Group 2 After CKPT ($n = 14$) | Group 3 Healthy ($n = 19$) |
|---|---|---|---|
| Digital systolic (mm Hg) | 119 ± 21** | 123 ± 23** | 101 ± 14 |
| Brachial systolic (mm Hg) | 158 ± 19** | 140 ± 13** | 113 ± 11 |
| Brachial diastolic (mm Hg) | 86 ± 13* | 85 ± 8** | 74 ± 7 |

**Table 4.** Microcirculatory findings. CKPT, Combined kidney and pancreas transplantation; CBV, capillary blood cell velocity. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (differences as compared to group 3. Values within brackets are between groups 1 and 2)

| | Group 1 Before CKPT ($n = 20$) | Group 2 After CKPT ($n = 14$) | Group 3 Healthy ($n = 19$) |
|---|---|---|---|
| Resting CBV ($mm\,s^{-1}$) | 0.17 ± 0.13** | 0.19 ± 0.18** | 0.46 ± 0.35 |
| Peak CBV ($mm\,s^{-1}$) | 0.49 ± 0.27** | 0.59 ± 0.37* ($P < 0.05$) | 0.93 ± 0.45 |
| Time to peak CBV (s) | 10.6 ± 3.9** | 8.2 ± 2.0 ($P < 0.05$) | 7.3 ± 3.0 |
| voLD[a] (%) | 30 ± 18*** | 48 ± 14* | 57 ± 16 |
| Skin temperature (°C) | 27.6 ± 2.4*** | 29.7 ± 2.5 | 31.0 ± 3.1 |

[a] % decrease of laser Doppler signal during venous occlusion

and 3 ($P < 0.05$). Skin temperature was lower in group 1 ($P < 0.001$) and more normal in group 2 than that in group 3.

## Discussion

Earlier investigations by our group have shown impaired regulatory responses of skin microcirculation in type 1 diabetics as compared to healthy controls [11, 22, 23, 28, 30, 31]. The time to peak capillary blood cell velocity (tpCBV) during postocclusive reactive hyperemia was found to be prolonged in diabetics with late complications [31], and the vasoconstrictor response to venous stasis was sometimes already impaired early after the onset of diabetes [23]. Following pancreas transplantation a sustained normoglycemia is obtained [32], but whether this normalization of the glucose homeostasis can delay or stop the progression of diabetic microangiopathy is unclear, even if some studies do point in this direction [1, 2]. In accordance with our earlier findings in patients with severe, late diabetic complications, the present study showed a significant impairment of all microvascular parameters in the pretransplantation group. The post-transplantation group also showed an impaired microvascular reactivity in most values as compared to the healthy controls, but a tendency towards better microvascular reactivity was found in the former group than in the pretransplantation group.

The rCBV was significantly reduced in both the pre- and post-transplantation groups as compared to the healthy controls. One would suspect that this reduction in rCBV was due to a lower skin temperature, but no correlation was found between skin temperature and rCBV. Several patients had a very low rCBV – even at high skin temperatures – which may be explained by a redistribution of blood from the nutritional skin capillaries to the subpapillary vascular beds. This may be brought about by a combination of an increased tone in the precapillary arterioles, e.g., due to hypertension [26] and structural changes [13], and an opening of the arteriovenous shunt vessels in the subpapillary, thermoregulatory vascular bed [6]. Such a redistribution of blood has also been shown to exist in diabetic patients in previous studies [3].

The reactive hyperemia response after an arterial occlusion is supposed to be regulated by a relaxation of the smooth muscle cells of the vessel wall and the influence of vasodilating metabolites produced by the ischemia during the arterial occlusion [7, 15]. After short occlusions, e.g., of 1 min, which was the time used in the present study, the myogenic factor is thought to be the predominating cause for the reactive hyperemia response, and metabolites in fact only play a role at longer occlusions [7, 15]. A delayed hyperemic response after arterial occlusion has been shown in patients with polycythemia [21]. However, the disturbed reactive hyperemia response found in the diabetic patients in this study cannot be explained by polycythemia since all patients were more or less anemic. The significantly reduced peak CBV and prolonged time to peak CBV during the reactive hyperemia that was found in the pretransplantation group may be explained by structural changes and/or a high vascular tone in the precapillary arterioles, hindering the relaxation of the smooth muscle cells in the vascular wall that normally occurs during a postocclusive reactive hyperemia. The normal time to peak CBV in the post-transplantation group indicates an improved ability to relax smooth muscle in this group. Even if the microvascular reactivity is better in the post-transplantation group, it is still partially impaired as compared to that of healthy controls. This may be due to the hypertension that still exists in these patients, but also to the fact that they were treated with both immunosuppressive agents and beta-blocking drugs, which may negatively influence the reactive hyperemia response.

The ability to decrease blood flow during venous stasis – the so called venoarteriolar reflex [14] – was strongly impaired in the pretransplantation group, whereas the post-transplantation group showed a better reaction. The reflex is supposed to prevent hyperfiltration in the capillary bed when the venous pressure rises [18], and it has been suggested that the mechanism behind this reflex is dependent on an intact sympathetic nerve activity [14]. Normalized blood glucose levels and improved kidney function could be factors positively influencing the tendency to the better venoarteriolar reflex seen in the post-transplantation group. Solders et al. [27] have recently suggested that the improved peripheral nerve function in diabetics after CKPT is mainly due to the elimination of uremia. However, it is doubtful whether the improvement in renal function after combined transplantation can explain our findings of a better reaction to venous stasis, as

preliminary results from studies in diabetic patients who had only undergone kidney transplantation show a marked impairment of microvascular reactivity in spite of an improved kidney function [16].

In the present study, we have not investigated the same patients before and after CKPT, but the results show a tendency towards better regulatory responses of skin microcirculation in diabetic patients 20 months after CKPT than in an equally disordered diabetic group awaiting CKPT. This may indicate that the normalized blood glucose homeostasis and the improved kidney function after CKPT can institute a regression, or at least stop the progression, of functional microvascular disturbances in skin microcirculation. However, some of the patients in this study may have passed the stage at which the diabetic microangiopathy can be reversed by the normalization of glucose metabolism. The observation period may also be too short for recovery of the damage, since it has been shown that it may take at least 2 years of near normoglycemia before any effect on diabetic retinopathy or incipient nephropathy can be observed [4, 17].

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