

Measurements of Oxygen Tension in Native and Transplanted Rat Pancreatic Islets

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This study was performed to measure the oxygen tension before and after revascularization of pancreatic islets transplanted beneath the renal capsule and to investigate to what extent this was affected by acute and chronic hyperglycemia. In addition, the oxygen tension in islets within the pancreas was determined. P_{O_2} was measured with a modified Clark electrode (tip 2–6 μm o.d.). Within native pancreatic islets, the mean P_{O_2} was higher (31–37 mmHg) than within the exocrine pancreas (20–23 mmHg). The mean oxygen tension in the transplanted islets the day after implantation was half of that recorded in native islets (14–19 mmHg) and did not differ between normoglycemic and diabetic recipients. At 1 month after transplantation, when revascularization had occurred, the mean P_{O_2} in the islet grafts was 9–15 mmHg in normoglycemic animals but was lower (6–8 mmHg) in diabetic animals, whereas the blood perfusion of the transplants, as measured with laser-Doppler flowmetry (probe diameter 0.45 mm), was similar in both groups. The mean oxygen tension in the superficial renal cortex surrounding the implanted islets was similar in all groups and remained stable at 13–21 mmHg. Intravenous administration of D-glucose (1 g/kg) did not affect the oxygen tension in any of the investigated tissues. We conclude that the mean P_{O_2} in islets implanted under the renal capsule is markedly lower than in native islets, not only in the immediate posttransplantation period but also 1 month after implantation, i.e., when revascularization has occurred. Furthermore, persistent hyperglycemia in the recipient leads to a further decrease in graft oxygen tension. To what extent this may contribute to islet graft failure is at present unknown. *Diabetes* 47:1027–1032, 1998

Pancreatic islets have a rich blood supply, which is of crucial importance for the delivery of oxygen and nutrients to the islet cells and for the dispersal of the secreted hormones to their target organs (1). When islets are isolated from the pancreas for in vitro culture and subsequent transplantation, their vasculature dedifferentiates or degenerates (2,3). This means that these islets are supplied with oxygen and nutrients solely by diffusion from

the medium or, immediately after transplantation, from the surrounding tissues. Furthermore, oxygen consumption of individual islet cells establishes oxygen concentration gradients, with a lower P_{O_2} in the β -cell-rich core than in the islet periphery (3,4).

The first few days after islet transplantation are characterized by substantial islet cell dysfunction and death, a process that is even more pronounced in diabetic recipients (5). Revascularization of the transplanted islets occurs rapidly and is completed within 7–14 days posttransplantation (6,7). At 4 weeks after transplantation, the blood perfusion of islets grafted beneath the renal capsule of normoglycemic rats is within the same range as that in native islets in the pancreas (8). In diabetic recipients, the blood flow of the transplanted islets seems to be lowered (8), which has been assumed to be caused by an altered regulation of the blood flow rather than a defect in the revascularization process (9,10).

Islet hypoxia is generally thought to be the major reason for the vulnerability of islets in the first few days of engraftment, although it has never been unequivocally demonstrated. The aim of the present study, therefore, was to measure P_{O_2} in native pancreatic islets and before and after revascularization of islets transplanted under the renal capsule. We also investigated to what extent graft oxygen tension was affected by acute and chronic hyperglycemia in the recipient. Furthermore, P_{O_2} within the grafts was correlated to the blood flow of the transplant by measuring blood perfusion with laser-Doppler flowmetry, and it was also correlated to blood glucose concentrations and graft insulin content.

RESEARCH DESIGN AND METHODS

Animals. Male inbred Wistar-Furth rats, weighing 300–350 g, were used in all experiments. The animals were purchased from Møllegaard Breeding and Research Center (Skensved, Denmark). They had free access to water and pelleted rat food throughout the study. All experiments were approved by the local animal ethics committee at Uppsala University.

Measurements of oxygen tension in native islets. Animals were anesthetized with an intraperitoneal injection of thiobutabarbital sodium (Inactin, 120 mg/kg body wt; Research Biochemicals International, Natick, MA), placed on a heated operating table maintained at body temperature (37.5°C), and tracheostomized. Polyethylene catheters were inserted into the right femoral artery and right femoral vein. The former catheter was connected to a Statham P 23dB pressure transducer to continuously monitor the mean arterial blood pressure, whereas the latter catheter was used to infuse Ringer solution (5 ml · h⁻¹ · kg⁻¹) to substitute for body fluid loss and for administration of D-glucose or 3-O-methylglucose.

The pancreas was exposed, immobilized, and its islets visualized in the same manner as previously described for measurements of islet capillary pressure (11). Briefly, the abdomen was opened by a midline incision, and the pancreas placed loosely over a hollow cylindrical plastic block, attached to the operating table, for immobilization. The pancreas preparations were then continuously superfused with mineral oil (Apoteksbolaget, Gothenburg, Sweden) at body temperature to prevent desiccation of the tissues. During the course of the experiment, the temperature of the preparation was monitored by a thermocouple probe (CT D85; ELLAB, Copenhagen, Denmark).

After allowing the mean arterial blood pressure to stabilize, 0.8 ml sterile-filtered 2% (wt/vol) neutral red (Kebo Grave, Stockholm, Sweden) was injected intra-

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ANOVA, analysis of variance; H&E, hematoxylin and eosin; PLSD, protected least-squares difference; STZ, streptozotocin; TPU, tissue perfusion units.

venously to selectively stain the islets within the pancreas (11). The dye has previously been shown not to affect blood glucose, serum insulin, or pancreatic or islet blood flow (11). After this, the animals were allowed to rest for at least 30 min to minimize the influence of surgical stress and neutral red administration on the oxygen tension measurements.

Oxygen tension in the islets and in the exocrine pancreas was measured by modified Clark microelectrodes (12,13), with a small tip diameter of 2–6 μm o.d. and 1–2 μm i.d. The electrodes were polarized at -0.800 V , which gave a linear response between the oxygen tension and the electrode current. The electrical current was measured by picoamperemeters (University of Aarhus, Aarhus, Denmark). The electrodes were calibrated in water saturated with N_2 gas or air at 37°C before and after the experiments. The drift of the microelectrodes recordings was $<0.5\%/h$.

The microelectrode tip was inserted into the endogenous islets and the exocrine pancreas by the use of a micromanipulator under a stereomicroscope. Measurements were performed in 3–6 islets of each animal. Multiple measurements of oxygen tension were usually performed within the same islet; the mean of all these measurements was calculated to obtain the oxygen tension value for one islet. The mean of the islet oxygen tension values in one animal was then considered to be one experiment. Oxygen tension measurements were performed both before and after an intravenous injection of 1.0 ml 30% (wt/vol) D-glucose or the nonmetabolizable glucose derivative 3-O-methylglucose. Blood glucose concentrations were determined with test reagent strips (Medisense; Baxter Travenol, Deerfield, IL) from samples obtained from the cut tip of the tail. In addition to the oxygen tension in the pancreas, the blood pressure, body temperature, and pancreatic temperature were continuously recorded with a MacLab Instrument (AD Instruments, Hastings, U.K.) connected to a Macintosh Power-PC 6100.

Islet isolation and transplantation. Pancreatic islets were prepared by collagenase (Boehringer-Mannheim, Mannheim, Germany) digestion, as described in detail elsewhere (14). Groups of ~150 islets were cultured free-floating for 4–7 days in RPMI 1640 medium supplemented with 10% vol/vol calf serum (Sigma, St. Louis, MO) (14), and the medium was exchanged every second day. After culture, 250 islets were packed in a braking pipette and then implanted beneath the capsule on the dorsal side of the left kidney in pentobarbital-anesthetized (60 mg/kg i.p.; Apoteksbolaget, Göteborg, Sweden) rats. Some of the recipients were injected with streptozotocin (STZ, 55 mg/kg i.v.) (Sigma) 4–7 days before transplantation and were diabetic (blood glucose concentration $>10\text{ mmol/l}$) at the time of transplantation. The number of islets transplanted was insufficient to reverse hyperglycemia in the STZ-induced diabetic rats.

Measurements of oxygen tension and blood flow in transplanted islets. At 1 day or 1 month after islet transplantation, the animals were anesthetized with thiobutobarbital, placed on a heated operating table, and tracheostomized as described above. Polyethylene catheters, also as given above, were inserted into the femoral artery and vein and used for recording mean arterial blood pressure and for substituting body fluid loss with Ringer solution, respectively.

The left kidney was exposed by a left subcostal flank incision and immobilized in a plastic cup. The kidney was embedded in pieces of cotton wool soaked in Ringer solution, and its surface was covered with mineral oil (Apoteksbolaget) to prevent evaporation and thereby keep the kidney surface continuously moist and at body temperature. During the course of the experiments, the temperature of the kidney was monitored by a thermocouple probe (CT D85). To avoid any confounding factors caused by urinary stasis, the left ureter was catheterized. The animals were then allowed to rest for at least 30 min to minimize the influence of surgical stress on blood flow and oxygen tension values.

In each animal, oxygen tension was measured in the islet graft and the adjacent renal parenchyma at different depths from the tissue surface (0, 250, 500, 750, and 1,000 μm). At least three measurements were performed at each depth, using modified Clark electrodes (see above). In the kidney of some of the animals, measurements were also performed at depths of 1,250 and 1,500 μm from the tissue surface. The mean of all measurements, in each tissue and animal, was calculated and considered to be one experiment. The mean oxygen tension values were also calculated separately for each investigated depth.

In the nondiabetic animals, measurements of oxygen tension, as described above, were performed both before and after intravenous administration of 1.0 ml of 30% (wt/vol) D-glucose or 3-O-methylglucose. In separate experiments, the microelectrodes were replaced by empty outer microelectrode casings with the same shape and size and inserted at the depths of 1,000, 1,250, or 1,500 μm from the islet tissue surface. After injection of a small amount of sterile-filtered 2% (wt/vol) neutral red (Kebo Grave), the outer casings were removed and the graft and kidney sectioned to morphologically evaluate the injection sites. All dye injections at the depth of 1,000 μm , and most of those at the depth of 1,250 μm , were located within the islet grafts of both control and diabetic recipients.

In conjunction with the oxygen tension measurements, the blood perfusion of the graft and the adjacent outer renal cortex was measured by laser-Doppler flowmetry (PF 4001-2; Perimed, Stockholm, Sweden) with a needle probe (411, tip 0.45 mm o.d.; Perimed) 1 month after implantation. Previous studies (7), as well as measurements of graft blood perfusion 1 day after transplantation in the pres-

ent study (P.O.C., P.L., A.A., L.J., unpublished observations), have demonstrated that values obtained before any adequate revascularization has taken place are associated with methodological errors making the results difficult to interpret. We therefore chose to study only animals transplanted 1 month before the blood flow measurements. Care was taken to position the flow probe perpendicular to the immobilized tissue surface by the use of a micromanipulator, without causing any compression of the tissue. At least three measurements of the blood perfusion of the transplanted islets and the adjacent renal cortex were performed for each tissue and animal. The mean of these measurements in each animal was calculated and considered to be one experiment. All recordings were performed under stable illumination. Because it is difficult to calibrate the instrument in physical units of blood flow, all blood flow values are given as arbitrary tissue perfusion units (TPU). In the nondiabetic animals, measurements of blood flow were performed both before and after intravenous administration of 1.0 ml of 30% (wt/vol) D-glucose or 3-O-methylglucose. To exclude any possible influence of the oxygen tension measurements on tissue blood flow, islet and renal blood flow in some animals was investigated both before and after measurements of oxygen tension. Blood glucose concentrations were determined with test reagent strips (Medisense), as described above.

Insulin content of islet grafts. After measuring the oxygen tension and blood flow, the islet grafts of some animals were dissected free and placed in 1.0 ml acid-ethanol (0.18 mol/l HCl in 95% [vol/vol] ethanol). The grafts were sonicated to disrupt the islet cells, and the samples were then extracted overnight at 4°C , followed by radioimmunological assay of the insulin contents (15).

Light microscopic evaluation and determination of islet graft volume. After the oxygen tension and blood flow measurements, the left kidneys in some of the animals were removed, fixed in 10% (vol/vol) formaldehyde for 24 h, transferred to 70% (vol/vol) ethanol, and embedded in paraffin. Sections (7 μm) were stained with hematoxylin and eosin (H&E) and evaluated for possible inflammatory changes or diabetes-induced changes in the recipient kidney by an observer unaware of the origin of the slides.

Separate nondiabetic and diabetic animals (STZ-induced) were transplanted with 250 islets beneath the renal capsule as described above. These grafts were excised with at least a 3-mm margin 1 month after transplantation, fixed in formaldehyde, and embedded in paraffin. Consecutive sections (12 μm thick) were stained with H&E. The total graft volumes and the fractions constituting endocrine cells were estimated by using a computerized system for morphometry (MOP-Videoplan; Carl Zeiss, Svenska, Stockholm, Sweden) as described elsewhere (8).

Statistical analysis. All values are given as means \pm SE. When multiple comparisons between data were performed, analysis of variance (ANOVA) and Fisher's protected least-squares difference (PLSD) test were used (Statview; Abacus Concepts, Berkeley, CA). When only two groups were compared, probabilities (P) of chance differences between the experimental groups were calculated using paired two-tailed Student's t test when comparing values from the same animal.

RESULTS

Blood glucose and arterial blood pressure. The mean blood glucose concentration was $5.0 \pm 0.1\text{ mmol/l}$ ($n = 41$) in the nondiabetic animals at the time of killing. An intravenous injection of 1.0 ml D-glucose increased the blood glucose levels to $16.0 \pm 0.6\text{ mmol/l}$ ($n = 21$) 15 min later, whereas administration of 3-O-methylglucose or neutral red did not influence the blood glucose concentrations (data not shown). The blood glucose concentrations of the STZ-induced diabetic animals were $15.4 \pm 0.8\text{ mmol/l}$ ($n = 13$) on the day of islet transplantation, $16.0 \pm 0.9\text{ mmol/l}$ ($n = 7$) the day after, and $25.5 \pm 1.2\text{ mmol/l}$ ($n = 6$) 1 month after transplantation. The animals were purposely given a graft, the size of which was not sufficient to reverse hyperglycemia in diabetic recipients.

The mean arterial blood pressure of all transplanted animals was $\sim 120\text{ mmHg}$ and did not differ between diabetic and nondiabetic animals. In animals in which the measurements of oxygen tension were performed in endogenous islets, the mean arterial blood pressure was slightly lower (105–110 mmHg).

Graft volume and insulin content. The islet transplants in the diabetic animals had a lower insulin content than grafts in nondiabetic recipients 1 day after implantation (Table 1). The insulin content was further decreased 1 month after transplantation in grafts of diabetic recipients (Table 1). However, the insulin content of the islet grafts in the nondiabetic recip-

TABLE 1
Insulin content of 250 islets syngeneically transplanted under the left renal capsule in male Wistar-Furth rats

	Insulin content (ng/graft)	
	Nondiabetic rats	Diabetic rats
1 day	4,394 ± 189 (14)	3,287 ± 324* (7)
1 month	5,444 ± 274§ (14)	2,062 ± 298†‡ (7)

Data are means ± SE (number of experiments). The grafts were analyzed 1 day or 1 month after transplantation to normoglycemic or hyperglycemic (STZ-induced diabetic) recipients. * $P < 0.01$ and † $P < 0.001$ when compared with nondiabetic recipients; ‡ $P < 0.05$ and § $P < 0.01$ when compared with posttransplantation day 1 using ANOVA and Fisher's PLSD test.

ipients had increased by ~25% 1 month posttransplantation (Table 1). The islet graft volume was similar in diabetic and nondiabetic animals when investigated 1 month after transplantation (308 ± 37 vs. 283 ± 16 nl; $n = 6$ in both groups). Connective tissue constituted ~20% of the graft in all recipients.

Tissue oxygen tension. At the surface of native pancreatic islets, the mean tissue oxygen tension varied between 37 and 46 mmHg, whereas the mean P_{O_2} within the islets tended to be somewhat lower, viz, 31–37 mmHg (Table 2). These values were not affected by administration of either D-glucose or 3-*O*-methylglucose (Table 2). The surface of the exocrine pancreas had a higher mean P_{O_2} than the interior parts of the gland (Table 2). Tissue oxygen tension was markedly higher within the islets than within the exocrine parenchyma (Table 2).

P_{O_2} did not differ between central and peripheral parts of the 1 day islet grafts (Fig. 1). In the islet transplants examined after 1 month, the highest values for tissue oxygen tension were, however, regularly recorded at the graft surface in both nondiabetic and diabetic animals (Fig. 1). Otherwise, no differences in oxygen tension within the grafts were seen 1 month postimplantation (Fig. 1). No correlation between P_{O_2} and the distance from the surface could be seen in the outer renal cortex (data not shown). Neither D-glucose nor 3-*O*-methylglucose affected the oxygen tension at any of the investigated depths in the 1-day and 1-month islet grafts (data not shown).

At 1 day after transplantation, the mean oxygen tension in islets transplanted beneath the renal capsule was markedly lower than in native islets (Table 3). At this time point, there

were no statistically significant differences in oxygen tension between nondiabetic and diabetic recipients (Table 3). At 1 month posttransplantation, the mean oxygen tension in the islet grafts was further decreased, but the difference was not statistically significant for the nondiabetic recipients if the surface values were included in the means (Table 3, Fig. 1). In the diabetic recipients, the oxygen tension values in the islet grafts were markedly lower than in the corresponding nondiabetic animals, irrespective of whether the surface values were included in the means or not (Table 3). Oxygen tension values in the superficial renal cortex adjacent to the grafts were similar in all groups at both 1 day and 1 month after implantation (Table 3).

Blood flow measurements. At 1 month after transplantation, the blood perfusion of the transplanted islets, measured with laser-Doppler flowmetry, was ~60% of that in the renal cortex (129 ± 13 vs. 224 ± 25 TPU in the grafts and kidney; $n = 7$ and 6, respectively). Neither D-glucose nor 3-*O*-methylglucose affected the graft blood flow (data not shown). There were no differences in the blood perfusion of the recipient kidneys (data not shown) or islets implanted into diabetic or nondiabetic recipients (151 ± 16 vs. 129 ± 13 TPU; $n = 6$ and 7, respectively). Blood flow in the outer renal cortex close to the graft was similar at 1 day and 1 month after islet transplantation (data not shown).

DISCUSSION

The immediate posttransplantation period is characterized by islet cell dysfunction and β -cell death (5). The reason for this is generally thought to be hypoxia, although it has never been proven by measurements of oxygen tension in islet grafts. The purpose of the present study, therefore, was to determine P_{O_2} before revascularization of pancreatic islets transplanted beneath the renal capsule and to compare this to values obtained in neovascularized islets at the same implantation site and to endogenous islets within the pancreas. Furthermore, the experiments were designed to make it possible to evaluate whether previously described detrimental effects of hyperglycemia on transplanted β -cells (5) are associated with a change in tissue oxygen tension.

P_{O_2} in islet implants was measured with a modified Clark electrode with a small tip diameter (2–6 μ m o.d.) and correlated to graft blood flow registered with laser-Doppler flowmetry. It has been shown that when oxygen tension is measured in tissues by polarographic oxygen microelec-

TABLE 2
Oxygen tension in native rat pancreatic islets and in the exocrine pancreatic parenchyma before and 15 min after administration of 1.0 ml 30% (wt/vol) D-glucose or 3-*O*-methylglucose

	Oxygen tension (mmHg)			
	D-Glucose treatment ($n = 7$)		3- <i>O</i> -methylglucose treatment ($n = 7$)	
	Before treatment	After treatment	Before treatment	After treatment
Islet surface	44.7 ± 2.8	45.7 ± 2.0	37.0 ± 2.3	41.5 ± 2.7
Islet interior	36.9 ± 2.6	31.7 ± 4.1§	31.0 ± 1.1	35.9 ± 1.7
Exocrine surface	35.0 ± 4.3	32.8 ± 6.0*	35.1 ± 1.2	34.9 ± 2.3
Exocrine interior	19.9 ± 2.9†	19.6 ± 5.0*§	22.0 ± 3.8*¶	23.1 ± 3.5‡

Data are means ± SE. * $P < 0.05$, † $P < 0.01$, and ‡ $P < 0.001$ when compared with the corresponding measurements in the islets; § $P < 0.05$, || $P < 0.01$, and ¶ $P < 0.001$ when compared with the corresponding measurements at the tissue surface using ANOVA and Fisher's PLSD test.

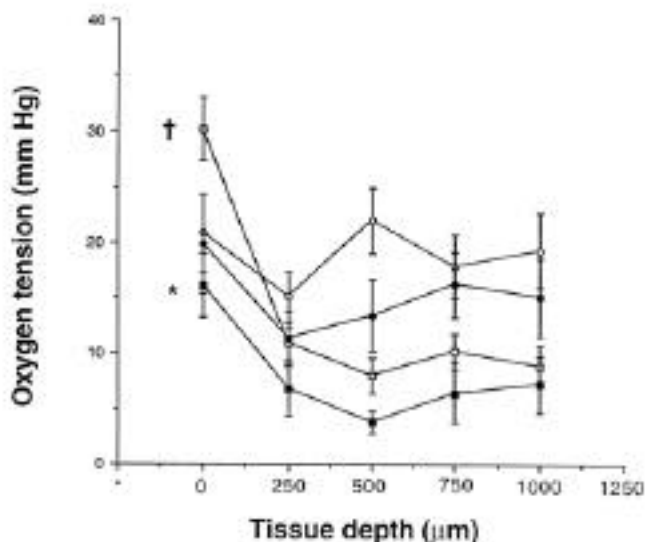


FIG. 1. Oxygen tension at different depths from the tissue surface in syngeneic islet grafts implanted under the renal capsule of control ($n = 14$; \circ, \square) and STZ-induced diabetic ($n = 6-7$; \bullet, \blacksquare) male Wistar-Furth rats at day 1 (\circ, \bullet) and 1 month (\square, \blacksquare) after transplantation. Values are means \pm SE. * $P < 0.05$ and † $P < 0.001$ when comparing surface values to values measured within the transplants with paired Student's t test.

trodes, the errors in these determinations are influenced to a large extent by the size of the electrode tip. Large electrodes (tip diameter $>10 \mu\text{m}$) decrease Po_2 by damaging tissues and compressing capillaries (16). Furthermore, large electrodes have a high diffusion current, reflecting a high oxygen consumption (17). The miniaturized Clark electrodes used in the present study were initially applied for environmental research (12) and have recently been further developed and modified for in vivo measurements of oxygen tension (13). A major advantage with this electrode is that all oxygen diffusing toward the sensing cathode is removed from the internal electrolyte reservoir by the guard cathode, placed 200–600 μm behind the sensory cathode. Furthermore, the small tip diameter (2–6 μm o.d.) minimizes tissue distortion.

Po_2 within endogenous islets in the pancreas was markedly higher than that within the exocrine pancreas and the kidney. This is in accordance with the high metabolic activity of islet

endocrine cells (18), the denser microvasculature of the islets (19), and the high basal islet blood perfusion (1). The administration of glucose did not affect the oxygen tension of the islets. This is probably due to the increase of islet blood flow induced by acute hyperglycemia (1), which compensates for the increased consumption of oxygen.

One consideration when performing measurements of Po_2 in transplanted islets is the exact placement of the tip of the electrode, i.e., whether it reflects the oxygen tension in aggregates of endocrine cells or within interspersed strands of connective tissue. Morphological examinations demonstrated that ~20% of the transplants consisted of stroma. In view of this, it seems likely indeed that some of the measurements of oxygen tension were made in parts of the grafts consisting of nonendocrine cells. However, in view of the large number of measurements performed in each graft, the error introduced by this is likely to be small. Furthermore, if there were any pronounced differences in the oxygen tension in different parts of the graft, we would have observed a bimodal distribution of our measurements of Po_2 , and this was not the case (data not shown).

At 1 day after transplantation, the oxygen tension within the islet grafts was ~15–20 mmHg, i.e., 50% of that in native islets and slightly lower than that in the adjacent renal cortex. This partial pressure was unaffected by exposure to acute or chronic (1 d) hyperglycemia. The reason for the surprisingly high oxygen tension, despite the lack of blood vessels within the graft, is likely due to the presence of fluid-filled spaces between the transplanted islets. It is to be anticipated that no organization of stromal tissues has occurred at this time point. The capacity for oxygen to diffuse in these spaces between the individual islets is unknown but is probably high. Furthermore, a posttraumatic increase in blood flow and blood vessel permeability in the adjacent renal cortex, which is likely present the day after transplantation, will increase the availability of oxygen. It can also be speculated that there is decreased oxygen consumption by individual islet cells because of decreased insulin production in the graft after the trauma associated with implantation.

Somewhat surprisingly, the oxygen tension in transplanted islets was lower 1 month after implantation than after 1 day, i.e., at a time point when revascularization is likely to be complete. This means that transplanted islets are exposed to

TABLE 3

Oxygen tension in syngeneic islet grafts transplanted beneath the renal capsule 1 day or 1 month before measurements and in the adjacent kidney cortex of diabetic or nondiabetic rats

	Oxygen tension (mmHg)			
	1 day after transplantation		1 month after transplantation	
	Nondiabetic recipient	Diabetic recipient	Nondiabetic recipient	Diabetic recipient
Islet (ws)	18.8 \pm 2.2 (14)	15.3 \pm 2.1 (7)	14.8 \pm 1.3 (14)	8.0 \pm 1.1 (6)*†
Islet (wos)	18.3 \pm 2.2 (14)	14.1 \pm 2.4 (7)	9.7 \pm 1.0 (14)‡	6.0 \pm 1.1 (6)*†
Kidney (ws)	18.4 \pm 1.6 (14)	13.9 \pm 2.4 (7)	20.4 \pm 1.5 (7)	19.7 \pm 2.3 (6)
Kidney (wos)	18.1 \pm 1.3 (14)	13.9 \pm 2.3 (7)	18.3 \pm 2.0 (7)	18.2 \pm 2.6 (6)

Data are means \pm SE (number of experiments) with (ws) or without (wos) surface values included in the means. The mean of all oxygen tension measurements in the kidney or the islet graft of an untreated animal was considered to be one experiment. * $P < 0.05$ when compared with the corresponding nondiabetic group; † $P < 0.05$ and ‡ $P < 0.001$ when compared with day 1 after transplantation using ANOVA and Fisher's PLSD test.

an oxygen concentration that is considerably lower than that seen in the native pancreas. Whether, and to what extent, this contributes to graft dysfunction is at present unknown. It can be speculated, however, that it leads to a selected survival of more hypoxia-resistant β -cells (see below). This further decrease in islet graft oxygen tension may reflect the organization of the stroma, which constituted ~20% of the graft volume, into a more dense network of collagenous and reticular fibers that prevented diffusion of oxygen into the spaces between the islets. Instead, all oxygen transportation at this time point depends on the newly formed microvessels within the grafts. It has previously been demonstrated that these vessels are immature at this time point (20), and that they possess a blood flow regulation different from that of native islets (9,21). This may be due to an inadequate production of endothelial mediators, mainly nitric oxide, which is of crucial importance for islet blood flow regulation (cf. 22). It is therefore possible that the newly formed capillaries have a reduced capacity to transport oxygen to the islet graft when compared with the highly specialized vascular network of the endogenous islets (cf. 1), and that this explains the lower P_{O_2} seen in grafted islets. In confirmation of this, laser-Doppler flowmetry demonstrated a graft blood perfusion amounting to only 60% of that of the surrounding kidney. Whether the capillary system of the islet grafts matures with time, and thereby increases its capacity for oxygen transport to the endocrine cells, is at present unknown.

The reasons for the further decrease in oxygen tension seen in the 1-month grafts after persisting hyperglycemia are obscure. It should be noted that the grafts were of the same size in both groups of recipients, as measured by morphometric procedures. Furthermore, the degree of revascularization seemed to be the same as suggested by the presence of numerous capsular blood vessels overlying the graft. This was also reflected by a higher oxygen tension at the surface than within the transplants, which was not seen in the avascular grafts on the first day after implantation. Histological examinations also suggested that the number of blood vessels was similar in all grafts, although this was not quantitatively determined. This is in line with previous investigations showing that hyperglycemia per se has no adverse effects on revascularization of transplanted islets (10), but that hyperglycemia may instead affect the regulation of graft blood flow (21). However, no differences in transplant blood flow between normoglycemic and hyperglycemic recipients could be detected in the present study. The presence of large blood vessels overlying the grafts suggests, however, that there may be a higher blood perfusion in the peripheral regions of the transplant, even though no clear regional differences in oxygen tension could be observed. It should be noted that one major difference when comparing blood flow measurements with microspheres, as in the earlier studies, with those performed with laser-Doppler flowmetry is that the latter measures the whole blood perfusion, i.e., all moving blood cells, within the illuminated tissue volume (23,24). Arterially injected microspheres, on the other hand, measure the inflow of particles from the systemic circulation, which are more likely to represent nutritive blood flow responsible for oxygen delivery. Furthermore, microspheres may be shunted through the immature graft vessels without becoming entrapped, thereby avoiding detection with a concomitant underestimation of graft blood flow. Another factor that may be of importance for the decreased oxygen tension in the

hyperglycemic recipients may be that the increased delivery of metabolic substrates and therefore increased demands for insulin release in the diabetic animals cause an increased consumption of oxygen by the remaining β -cells.

No distinct gradients in P_{O_2} , suggesting the presence of more hypoxic conditions in the center of the transplant, were observed in any of the animals. These findings are in contrast to those seen *in vitro* when the oxygen tension in isolated Brockman bodies from a Teleost fish was investigated. In that study, an oxygen tension gradient with maximal values at the surface and considerably lower values at the center of the Brockman bodies was seen (25). However, it should be noted that these bodies were much larger and more compact than the grafts used in the present study.

The insulin content of the transplants was used to evaluate islet function in relation to the measured oxygen tension. Consistent with previous studies (5), the insulin content was decreased in islet transplants of diabetic animals 1 day after transplantation. The insulin content was further decreased in the diabetic animals when compared with normoglycemic animals 1 month after transplantation. We suggest that these detrimental effects of chronic hyperglycemia on long-term islet function may, at least partially, be explained by the more pronounced hypoxia to which islet cells are exposed in the diabetic environment. However, despite the decreased oxygen tension values recorded 1 month posttransplantation, the insulin content increased ~25% in the normoglycemic recipients when compared with day 1 after transplantation. The reason for this is unknown.

Interestingly, Dionne et al. (4) demonstrated that the second phase of insulin secretion was decreased by 50% at an oxygen tension of 27 mmHg in perfusion studies of freshly isolated rat islets. However, when islets had been precultured, a 50% decrease in insulin secretion was not seen until P_{O_2} was lowered to 10 mmHg. It was suggested that these findings were due to an early death of β -cells exposed to the lowest oxygen tension values, i.e., those located centrally in larger islets. In previous perfusion studies of islet-graft bearing kidneys in mice 4–12 weeks after islet implantation (26,27), we have failed to discern any disturbances in the second phase of glucose-stimulated insulin release. This may be because the remaining β -cells were probably less sensitive to hypoxia and/or located close to the newly formed blood vessels. This selective survival, and maybe growth, of the most hypoxia-resistant islet cells, as initially suggested by Dionne et al. (4), would then be able to maintain a normal second phase of insulin release despite the low tissue oxygen tension.

In summary, we have for the first time measured P_{O_2} in native islets and in islets transplanted beneath the renal capsule. The oxygen tension in the transplanted islets was markedly lower than that in native islets, also after revascularization. Our findings that chronic hyperglycemia worsened the extent of this hypoxia further underlines the necessity to keep glucose control as strict as possible in patients receiving an islet graft.

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