Markedly Decreased Oxygen Tension in Transplanted Rat Pancreatic Islets Irrespective of the Implantation Site

Per-Ola Carlsson,[1] Fredrik Palm,[2] Arne Andersson,[1] and Per Liss[3]

In this study, we syngeneically transplanted islets to three different implantation sites of diabetic and nondiabetic rats, then 9–12 weeks later we measured the blood perfusion and compared the tissue partial pressure of oxygen (Po_2) levels of these transplanted islets to endogenous islets. Modified Clark microelectrodes (outer tip diameter 2–6 μm) were used for the oxygen tension measurements, and islet transplant blood perfusion was recorded by laser-Doppler flowmetry (probe diameter 0.45 mm). The islet graft blood perfusion was similar in all islet grafts, irrespective of the implantation site. In comparison, the three implantation organs (the kidney cortex, liver, and spleen) differed markedly in their blood perfusion. There were no differences in islet graft blood perfusion between diabetic and nondiabetic recipients. Within native pancreatic islets, the mean Po_2 was ~40 mmHg; however, all transplanted islets had a mean Po_2 of ~5 mmHg. The oxygen tension of the grafts did not differ among the implantation sites. In diabetic recipients, an even lower Po_2 level was recorded in the islet transplants. We conclude that the choice of implantation site seems less important than intrinsic properties of the transplanted islets with regard to the degree of revascularization and concomitant blood perfusion. Furthermore, the mean Po_2 level in islets implanted to the kidney, liver, and spleen was markedly decreased at all three implantation sites when compared with native islets, especially in diabetic recipients. These results are suggestive of an insufficient oxygenization of revascularized transplanted islets, irrespective of the implantation site. *Diabetes* 50: 489–495, 2001

A factor of potential importance in the failure of islet grafts is poor or inadequate engraftment of the islets in the implantation organ. Normally, pancreatic islets have a dense glomerular-like capillary network in which the capillaries course through the islet in a tortuous fashion that is ideal for the delivery of oxygen and nutrients to the islet cells and for the dispersal of the secreted hormones to the target organs (1,2). This pancreatic islet angioarchitecture entails a blood perfusion of the pancreatic islets that is 10 times higher than that in the exocrine pancreas and similar to that seen in the renal cortex (~5–7 $ml \cdot min^{-1} \cdot g^{-1}$) (3–6). However, during the process of isolation and in vitro culture of pancreatic islets preceding transplantation, the islet vasculature dedifferentiates or degenerates (7,8). Therefore, immediately after transplantation, the pancreatic islets are supplied with oxygen and nutrients solely by diffusion from the surrounding tissues. The revascularization process is initiated within a few days, and the islets are generally thought to be fully revascularized by 1 month posttransplantation (9,10). We had previously observed markedly decreased oxygen tension in islets transplanted beneath the renal capsule at 1 month posttransplantation, a decrease that was even more pronounced in diabetic animals (11). Recently, we were able to show that this low islet graft tissue oxygen tension persists for ≥9 months after transplantation to the renal subcapsular site (12). A concomitant low islet graft blood perfusion could also be observed (11–13). It is not known whether this low oxygen tension and blood perfusion is a phenomenon specific for revascularized islets in the kidney subcapsular site or also applies to transplanted islets in other implantation sites. Therefore, the aim of the present study was to measure tissue oxygen tension in native islets and in islets transplanted under the liver, splenic, or left renal capsules, respectively. The oxygen tension in the islet transplants was determined 9–12 weeks after transplantation and correlated to the blood perfusion (by measuring graft blood perfusion with laser-Doppler flowmetry), to blood glucose concentrations, and to graft insulin content.

RESEARCH DESIGN AND METHODS

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

Islet isolation and transplantation. Pancreatic islets were prepared by collagenase digestion and were cultured free-floating in groups of ~150 islets for 4–7 days in RPMI-1640 medium supplemented with 10% vol/vol calf serum (Sigma, St. Louis, MO) (14). Medium was exchanged every second day. After culture, 250, 500, or 1,000 islets were packed in a braking pipette and implanted beneath the capsule of the liver, spleen, or left kidney in 60 mg/kg i.p. pentobarbithal–anesthetized (Apoteket, Göteborg, Sweden) rats. A small incision was made in the capsule of the implantation organ, allowing the braking pipette to be introduced. The pipette was then inserted 0.5–1 cm between the capsule structure and the organ parenchyma before the islets were gently ejected. This was done to ensure that none of the islets was lost through the incision hole. Some of the recipients were given 55 mg/kg i.v. streptozotocin (STZ) from Pharmacia-Upjohn (Kalamazoo, MI) 4–6 days

From the Departments of Medical Cell Biology[1], Physiology[2], and Diagnostic Radiology[3], Uppsala University, Uppsala, Sweden.

Address correspondence and reprint requests to Per-Ola Carlsson, Department of Medical Cell Biology, Biomedical Center, Husargatan 3, Box 571, SE-751 23 Uppsala, Sweden. E-mail: Per-Ola.Carlsson@medcellbiol.uu.se.

Received for publication 27 March 2000 and accepted in revised form 5 December 2000.

ANOVA, analysis of variance; STZ, streptozotocin; TPU, tissue perfusion units.

before transplantation and were diabetic (blood glucose >15 mmol/l) at the time of transplantation. The number of islets transplanted was purposely chosen to be insufficient (250–500) or sufficient (1,000) to reverse hyperglycemia in the STZ-administered animals. Blood glucose concentrations were determined with test reagent strips (Medisense; Baxter Travenol, Deerfield, IL) from samples obtained from the cut tip of the tail.

Measurements of oxygen tension in native and transplanted islets. The animals were anesthetized with thiobutabarbital sodium (Inactin; Research Biochemicals International, Natick, MA) at 120 mg/kg body wt i.p., placed on an operating table maintained at body temperature (37°C), and tracheostomized. Polyethylene catheters were inserted into the right femoral artery and the right femoral vein. The former catheter was connected to a pressure transducer (P23dB; Statham Laboratories, Los Angeles, CA) to monitor mean arterial blood pressure, whereas the latter catheter was used for continuous infusion (5 ml · kg^{-1} · h^{-1} body wt) of Ringer solution to substitute for loss of bodily fluid.

A left subcostal flank incision was made in animals with islets transplanted beneath the splenic or left renal capsule, and a midline incision with an extended right subcostal flank incision was performed in animals with islets implanted beneath the liver capsule. The organ with an islet graft was thereafter immobilized in a plastic cup attached to the operating table, embedded in pieces of cotton wool soaked in Ringer solution, with its surface covered with mineral oil (Apoteksbolaget, Gothenburg, Sweden) to prevent evaporation and to keep the tissue moist and at body temperature. During the course of the experiment, the temperature of the tissue surface was monitored by a thermocouple probe (CT D85; Ellab, Copenhagen). The animals were then allowed to rest for ≥30 min to minimize the influence of surgical stress on the measurements.

Oxygen tension was then measured in the islet graft and the adjacent tissue parenchyma with modified Clark microelectrodes (Unisense, Aarhus, Denmark) (11,15,16). The microelectrodes were polarized at −0.800 V, which gave a linear response between the oxygen tension and the electrode current; the latter was measured by picoamperemeters (University of Aarhus, Aarhus, Denmark). The electrodes (outer tip diameter 2–6 μm) were inserted into the tissues by the use of a micromanipulator under a stereomicroscope. The electrodes were calibrated in water saturated with $Na_2S_2O_5$ or air at 37°C. The drift of the microelectrodes recordings was <0.5% per h. In both the implantation organ (liver, spleen, or kidney) and the islet graft, at least 10 measurements were performed. The mean of all measurements in each tissue and animal was calculated and considered to be one experiment. Blood glucose concentrations were determined with test reagent strips (Medisense), as described above.

For oxygen tension measurements in native pancreatic islets, the pancreas was exposed and immobilized, and its islets were visualized; this was done in the same manner as previously described (6,11). Briefly, the abdomen was opened by a midline incision, and the pancreas was immobilized over a cylindrical plastic block attached to the operating table then superfused with mineral oil. Sterile-filtered neutral red (Kebo Grave, Stockholm) was administered at 0.8 ml 2% (wt/vol) i.v. to selectively stain the islets within the pancreas (6). This dye has previously been shown to not affect blood glucose, serum insulin, or pancreatic or islet blood flow (6). Moreover, in separate experiments, the partial pressure of oxygen (Po_2) in the exocrine pancreatic tissue was unaffected by neutral red (17). In each animal, measurements of oxygen tension were performed in 3–5 superficial pancreatic islets and in the surrounding exocrine parenchyma. Multiple measurements were usually performed within the same islet; the mean 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.

During the oxygen tension measurements, blood pressure, body temperature, and tissue temperature were continuously recorded with a MacLab Instrument (AD Instruments, Hastings, U.K.) connected to a Power Macintosh 6100 computer. Before killing the animals, arterial blood samples for analysis of hematocrit were obtained from the catheter in the femoral artery and collected in microhematocrit tubes (Kebo Grave, Stockholm). These samples were then centrifuged for 2 min at 13,000 rpm (Hettich Zentrifugen, Tuttlingen, Germany) to enable analysis.

Measurements of blood flow in transplanted islets. In conjunction with the oxygen tension measurements, the blood perfusion of the islet graft and the adjacent tissue (liver, spleen, or kidney) was measured by laser-Doppler flowmetry (PF 4001–2; Perimed, Stockholm) using a needle probe with a 0.45-mm external diameter tip (411; Perimed). The flow probe was positioned perpendicular to the immobilized tissue surface by the use of a micromanipulator, and care was taken not to cause any compression of the tissue. At least three measurements of the blood flow of the transplanted islets and the adjacent tissue were performed for each animal. The mean of these measurements in each animal was calculated and considered to be one experiment. 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).

Light microscopic evaluation. In some of the animals, the graft-bearing left kidneys were removed, fixed in 10% (vol/vol) formaldehyde for 24 h, transferred to 70% (vol/vol) ethanol, and embedded in paraffin. Sections 7 μm thick were stained with hematoxylin and eosin and used for evaluation of possible inflammatory changes or diabetes-induced changes in the islet graft and the recipient kidney. The light microscopic evaluation was performed by an observer unaware of the origin of the samples.

Graft insulin content. The islet grafts in most of the transplanted 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 (18).

Statistical analysis. All values are given as means ± SE. Multiple comparisons between data were performed using analysis of variance (ANOVA) (Statview; Abacus Concepts, Berkeley, CA), and P values were corrected using the Bonferroni method (19). For all comparisons, $P < 0.05$ was considered statistically significant.

RESULTS

Blood glucose and arterial blood pressure. The mean blood glucose concentration was 6.2 ± 0.1 mmol/l ($n = 30$) in the nondiabetic animals receiving 250 islets at the time of killing. All animals given STZ were diabetic (blood glucose >15 mmol/l) within 3 days after treatment. Despite similar blood glucose concentrations at the time of islet transplantation (18.6 ± 0.4, 18.7 ± 0.8, and 19.0 ± 0.9 mmol/l in animals diverted to implantation of 250 islets to the kidney, liver, or spleen, respectively), 9–12 weeks later, blood glucose levels were higher in animals with 250 islets implanted in the spleen or liver than in those with 250 islets implanted in the kidney (28.2 ± 1.4, 26.9 ± 0.6, and 22.1 ± 1.1 mmol/l and $n = 8$, 6, and 7; respectively; $P < 0.05$). These animals were purposely given a graft, which was not sufficient in size to reverse hyperglycemia in diabetic recipients. At the time of islet transplantation, animals receiving 500 or 1,000 islets had a mean blood glucose concentration of 19.8 ± 0.5 and 19.0 ± 0.3 mmol/l, respectively. At 9–12 weeks later, the blood glucose concentration in the animals having received 1,000 islets was 7.3 ± 0.2 mmol/l, whereas animals with grafts of 500 islets had a mean blood glucose level of 15.3 ± 0.4.

Mean arterial blood pressure was ~110 mmHg in all transplanted animals and did not differ between diabetic and nondiabetic recipients. In the animals in which Po_2 measurements were performed in endogenous islets, the mean arterial blood pressure was slightly lower (100–105 mmHg).

Hematocrit and graft insulin content. The hematocrit values recorded were similar in normo- and hyperglycemic animals (range 43–52). The transplants of 250 islets implanted beneath the renal or splenic capsule in diabetic animals had a lower insulin content than corresponding grafts in nondiabetic recipients (Table 1). However, in terms of the insulin content of 250 islets implanted beneath the liver capsule, the difference between diabetic and nondiabetic animals did not attain statistical significance (Table 1). The insulin content of islet grafts implanted in nondiabetic animals was similar irrespective of the implantation site (Table 1). Grafts consisting of 500 or 1,000 islets contained 1,782 ± 172 and 6,809 ± 1,192 ng insulin, respectively.

Islet transplant and implantation organ morphology. There were no signs of inflammatory reactions in either the islet transplants or the adjacent tissue of the implan-

TABLE 1
Insulin content of 250 islets syngeneically transplanted beneath the renal, hepatic, or splenic capsule in male Wistar-Furth rats 9–12 weeks before measurements

	Insulin content (ng/graft)	
	Nondiabetic rats	Diabetic rats
Kidney	3,793 ± 530	1,630 ± 247*
Liver	2,874 ± 534	1,668 ± 229
Spleen	2,893 ± 407	1,234 ± 142*

Data are means ± SEM for 6–11 experiments. All values are given for nondiabetic recipients and recipients with STZ-induced diabetes. There were no differences between the implantation organs of nondiabetic and diabetic recipients. *$P < 0.05$ vs. the corresponding nondiabetic animals. All comparisons were made with ANOVA.

tation organ (kidney, liver, or spleen) in any of the investigated animals. The morphology of the islet transplants and implantation organ was also similar in diabetic and nondiabetic animals, with connective tissue constituting <25% of the grafts.

Tissue oxygen tension. In native pancreatic islets, the tissue oxygen tension was ~40 mmHg (Fig. 1). In the transplanted islets, tissue Po_2 levels were markedly lower (Figs. 1,2). Similar values for tissue Po_2 were recorded in all transplanted islets, irrespective of the implantation organ (kidney, liver, or spleen). In the diabetic recipients, the tissue Po_2 level of islets transplanted beneath the renal and splenic capsule was even further decreased (Figs. 1,2). Islets transplanted beneath the liver capsule had tissue Po_2 levels similar to those of the corresponding nondiabetic animals (Fig. 1). STZ-administered animals transplanted with an islet mass sufficient to cure diabetes had levels of Po_2 in their graft tissue similar to those in nondiabetic animals with grafts consisting of 250 islets (Fig. 2). In the exocrine pancreas parenchyma, the Po_2 was ~30 mmHg (Fig. 3). In nondiabetic animals, a similar tissue oxygen tension was recorded in the splenic parenchyma, although the renal cortex, and especially the liver parenchyma, had a much lower tissue Po_2 level (Fig. 3). The splenic parenchyma of the diabetic rats displayed a lower Po_2 level than that found in the nondiabetic rats (Fig. 3). In contrast, no such difference was recorded in the kidney or the liver (Figs. 3,4).

Blood flow measurements. The blood perfusion of the transplanted islets was similar at all three implantation sites (kidney, liver, and spleen) (Fig. 5). Furthermore, there were no differences recorded in islet transplant

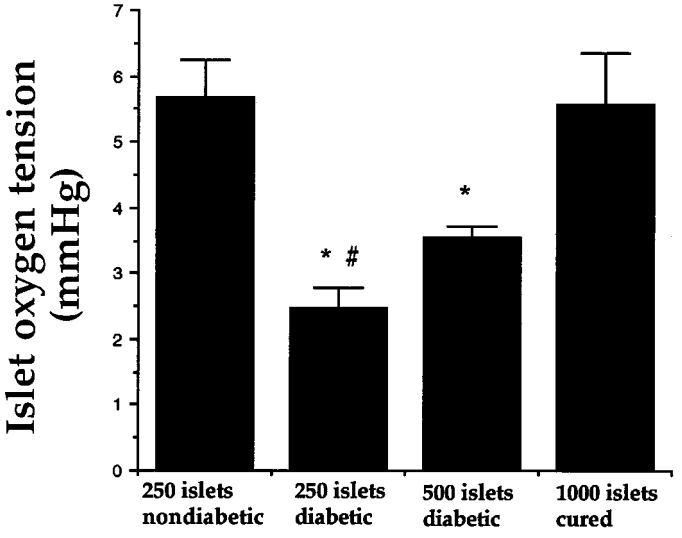

FIG. 2. Mean Po_2 levels in pancreatic islets (250, 500, or 1,000) transplanted beneath the renal capsule of syngeneic nondiabetic and diabetic Wistar-Furth rats. Animals with STZ-induced diabetes that were transplanted with 1,000 islets were cured of diabetes. Measurements were performed 9–12 weeks after transplantation. Note that Po_2 values for grafts consisting of 250 islets are also given in Fig. 1. All values are means ± SE for 7–8 animals. *$P < 0.05$ when compared with nondiabetic animals transplanted with 250 islets and #$P < 0.05$ when compared with cured diabetic animals (1,000 islets). All comparisons were made using ANOVA.

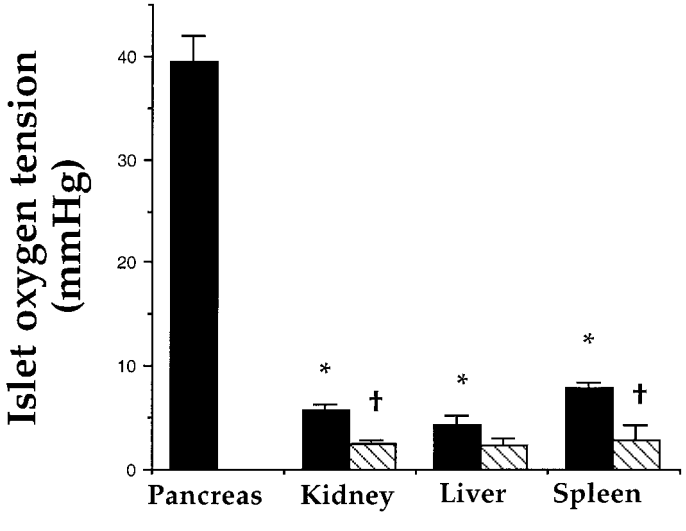

FIG. 1. Mean Po_2 levels in native pancreatic islets and in islets (250) transplanted beneath the renal, hepatic, or splenic capsule of syngeneic nondiabetic (■) and diabetic (▨) Wistar-Furth rats. Measurements were performed 9–12 weeks after transplantation. All values are means ± SE for 6–8 animals. *$P < 0.05$ when compared with native islets and †$P < 0.05$ when compared with the corresponding nondiabetic animals. All comparisons were made using ANOVA.

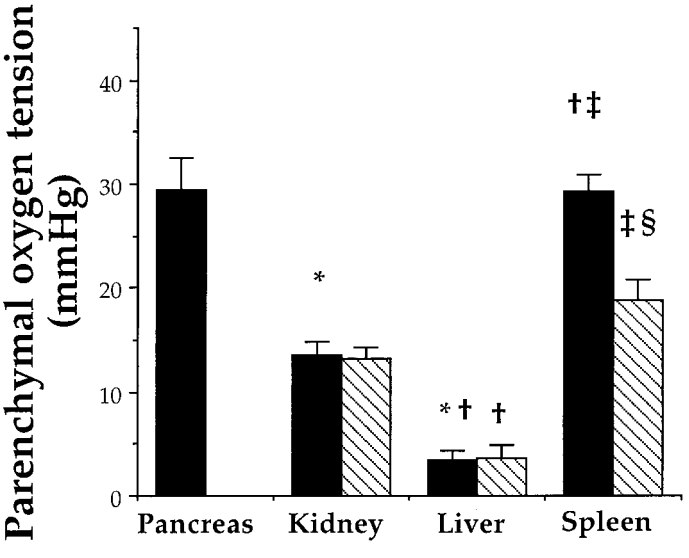

FIG. 3. Mean Po_2 levels in the exocrine pancreas parenchyma and in the parenchyma of the organs (kidney, liver, and spleen) receiving transplanted islets (250). ■, Nondiabetic Wistar-Furth rats; ▨, diabetic Wistar-Furth rats. All values are means ± SE for 6–8 animals. *$P < 0.05$ when compared with the exocrine pancreas; †$P < 0.05$ when compared with the renal cortex; ‡$P < 0.05$ when compared with the liver cortex; and §$P < 0.05$ when compared with the corresponding nondiabetic animals. All comparisons were made using ANOVA.

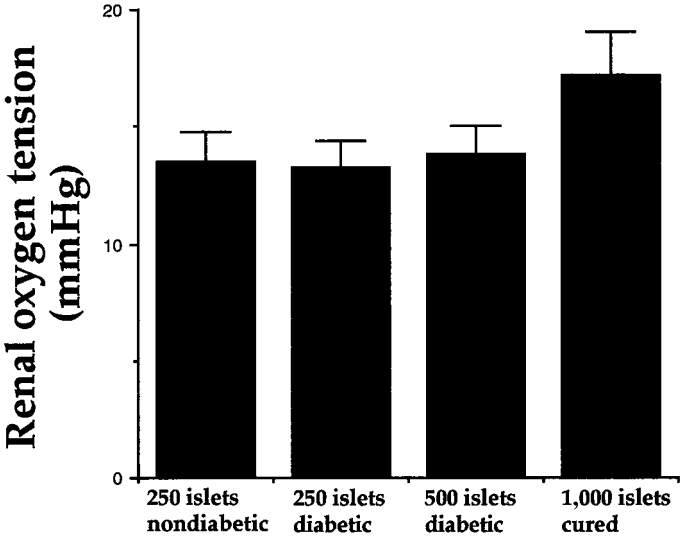

FIG. 4. Mean Po_2 levels in the superficial renal cortex adjacent to the transplanted pancreatic islets (250, 500, or 1,000) of nondiabetic and diabetic Wistar-Furth rats. Animals with STZ-induced diabetes that were transplanted with 1,000 islets were cured of diabetes. Note that Po_2 values for renal cortex adjacent to grafts of 250 islets also are given in Fig. 3. All values are means ± SE for 7–8 animals. All comparisons were made using ANOVA.

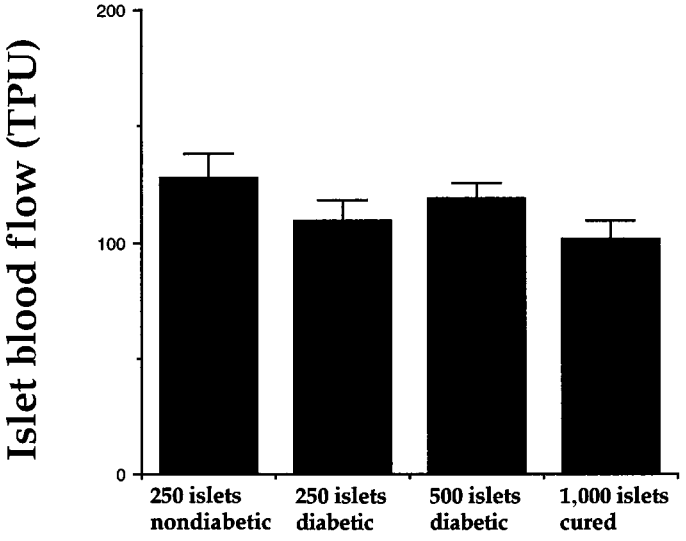

FIG. 6. Blood flow in islets (250, 500, or 1,000) transplanted beneath the renal capsule of syngeneic nondiabetic and diabetic Wistar-Furth rats. Animals with STZ-induced diabetes that were transplanted with 1,000 islets were cured of diabetes. Note that blood flow values for grafts consisting of 250 islets also are given in Fig. 5. Measurements were performed 9–12 weeks after transplantation. All values are means ± SE for 7–8 animals.

blood flow when comparing diabetic and nondiabetic recipients (Figs. 5,6). With regard to the blood perfusion of the organs bearing the islet grafts, the renal cortex had a higher blood perfusion than both the liver and splenic parenchyma in nondiabetic animals (Fig. 7). In the diabetic animals, the blood perfusion of the renal cortex was of the same order of magnitude as that of the liver parenchyma, whereas the spleen parenchyma had a markedly lower blood perfusion (Fig. 7). All three implantation organs had similar blood perfusion in diabetic and nondiabetic recipients (Fig. 7). STZ-administered animals transplanted with an islet mass sufficient to cure diabetes had renal blood flow similar to that of nondiabetic and diabetic animals with grafts consisting of 250 or 500 islets (data not shown).

DISCUSSION

With conflicting results, considerable research has been devoted to determining the most ideal implantation site for transplanted islets (20–24). In clinical practice, transplantation of pancreatic islets to the liver has been almost completely predominant, although in some cases the spleen has also been used as an implantation organ (25,26). Although adequate revascularization is of pivotal importance for optimal function after islet transplantation, there have been no studies comparing blood perfusion and oxygenization of islets transplanted to different implantation sites. In the present study, we transplanted islets to three different implantation sites (the kidney, liver, and spleen),

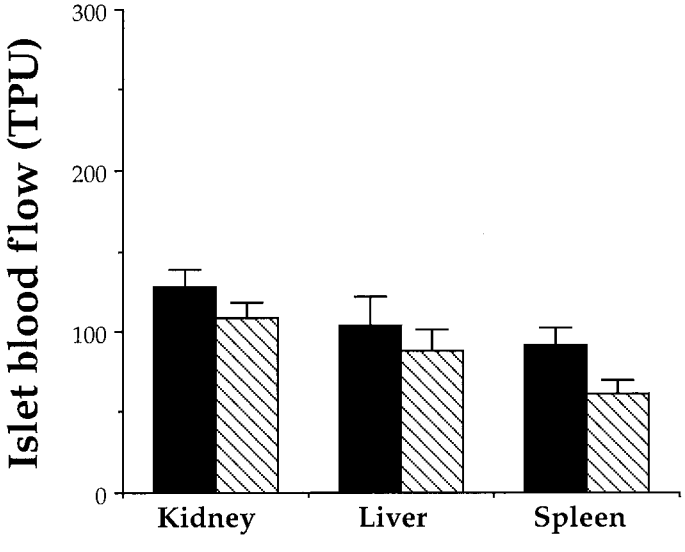

FIG. 5. Blood flow in islets (250) transplanted beneath the renal, hepatic, or splenic capsule of syngeneic nondiabetic (■) and diabetic (▨) Wistar-Furth rats. Measurements were performed 9–12 weeks after transplantation. All values are means ± SE for 6–8 animals.

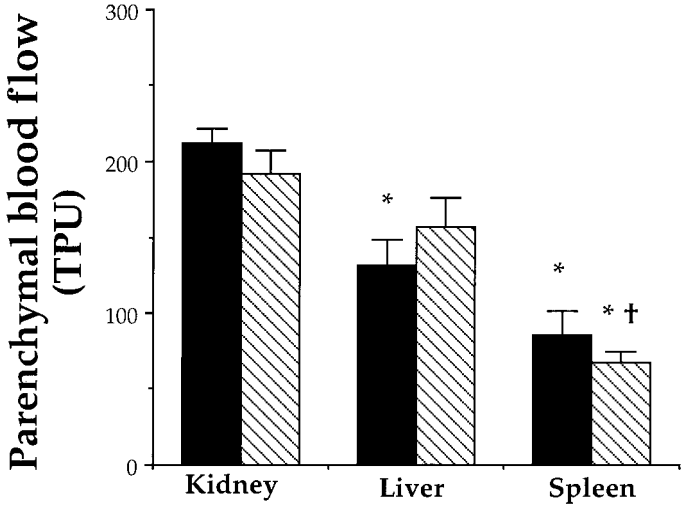

FIG. 7. Blood flow in the parenchyma of the organs receiving transplanted islets (250), i.e., kidney, liver and spleen, of nondiabetic (■) and diabetic (▨) Wistar-Furth rats. All values are means ± SE for 6–8 animals. * $P < 0.05$ when compared with the renal cortex and † $P < 0.05$ when compared with the liver. All comparisons were made using ANOVA.

and 9–12 weeks later we compared the tissue Po_2 levels and blood perfusion of these transplanted islets to that of endogenous islets. The islet graft blood perfusion was similar in all islet grafts, irrespective of the implantation site. In comparison, the three implantation organs (the kidney cortex, liver, and spleen) differed markedly in their blood perfusion. Therefore, a tentative conclusion is that, with regard to the degree of revascularization and concomitant blood perfusion, the choice of implantation organ is less important than the intrinsic properties of the transplanted islets. In other words, the transplanted islets, and not the implantation organ, seem to determine the degree of blood perfusion after revascularization. Interestingly, the transplanted islets also had markedly lower mean Po_2 levels than endogenous islets when investigated 9–12 weeks after transplantation, irrespective of the implantation site. In diabetic recipients, Po_2 levels in the transplanted islets tended to be even further decreased. The acquired level of blood perfusion in transplanted islets may therefore be inadequately low for optimal islet function, irrespective of the implantation organ.

Transplanted islets are generally considered to be rapidly revascularized, with no further vascular growth occurring after 1 month posttransplantation. Morphological (27,28) and in vivo microscopic studies (9) have suggested that islet grafts within this time span acquire a capillary angioarchitecture similar to that of native islets. Measurements of islet transplant blood perfusion in islets transplanted to the kidney subcapsular space also indicate a rapid revascularization, with no further increase in the blood perfusion occurring after the immediate posttransplantation period (7–14 days) (10,12). Morphological aberrances have been observed in the islet graft blood vessels, with 20% being devoid of an endothelial cell lining 6 weeks posttransplantation (29). Altered blood flow regulation of the newly formed graft blood vessels, compared with endogenous islets, has also been repeatedly described (11,30–32). This may be caused by inadequate production of endothelial mediators, which are of crucial importance for islet blood flow regulation (1). In native islets, nitric oxide seems especially important in maintaining the high basal islet blood flow of 5–7 ml $\cdot$ min^{-1} $\cdot$ g^{-1} (33,34). In the present study, laser-Doppler flowmetry demonstrated an islet graft blood perfusion amounting to only ~50% of the renal cortex blood flow and a blood perfusion similar to that of the spleen. In previous studies, we have also recorded similar low blood flow in islets transplanted to the renal subcapsular site using the laser-Doppler technique (11,12,32). It should be noted that laser-Doppler flowmetry measures whole blood perfusion (i.e., all moving blood cells) within the illuminated tissue. However, only capillary blood flow contributes to the delivery of oxygen to the cells. Indeed, in experiments using a combination of microspheres and an ultrasonic flow probe, a nutritional islet graft blood perfusion only ~10% of that seen in native islets was observed (13). An islet blood flow more similar to that of native islets has been recorded in islets autotransplanted beneath the renal capsule of partially pancreatectomized animals (30,31,35). However, in these latter experiments, the influence of partial pancreatectomy has been difficult to assess.

In terms of islet transplant blood perfusion, no differences were recorded between diabetic and nondiabetic animals at any of the implantation sites. The lower islet graft tissue oxygen tension observed in the diabetic animals—with significant difference in islets transplanted to the kidney and spleen—is thus likely to reflect other factors, such as increased consumption caused by increased demands for insulin release. The percentage of stroma in the grafts was similar in diabetic and nondiabetic animals, suggesting that occasional measurements in the stroma compartment of the grafts did not influence these observed differences. Transplantation of a larger islet mass (1,000 islets) to the kidney subcapsular site cured the animals with STZ-induced diabetes. These cured animals had islet graft blood perfusion and Po_2 levels similar to that of nondiabetic animals with remaining endogenous islets and grafts consisting of 250 islets. Therefore, any suppression of graft blood perfusion and subsequent islet graft oxygen tension induced by endogenous islets seems not to occur in these latter animals. Interestingly, diabetic animals transplanted with 500 islets had intermediate readings for blood glucose concentration and islet transplant Po_2 compared with diabetic animals transplanted with either 250 or 1,000 islets. This suggests that tissue oxygen tension in the islet transplants is directly related to blood glucose concentration. The size of the graft seems to be of less importance for tissue oxygen tension levels in view of the similar oxygen tension found in both the 250-islet graft in nondiabetic animals and the 1,000-islet graft in cured diabetic animals.

To maintain an intact oxygen consumption in mitochondria, an oxygen tension of 0.5–1 mmHg has been shown to be more than sufficient (36). Tissue oxygen tension levels similar or even lower than those recorded in the transplanted islets have also been shown repeatedly in living tissue (37–40). However, pancreatic β-cells normally have very high metabolic activity to meet the various demands for insulin secretion (41). The recorded Po_2 level may be sufficiently high throughout the transplanted islets to maintain cell viability, but there may be insufficient oxygen to support substantial insulin production and secretion. Transplanted islets in an environment of chronic hyperglycemia, with its extremely high demands for insulin secretion, may especially suffer from insufficient tissue oxygen tension. Interestingly, in a study by Papas et al. (42), increased lactate production was recorded from mouse insulinoma βTC3 cells already at a Po_2 level of 25 mmHg, whereas insulin secretion was gradually affected <7 mmHg. Furthermore, in perifused cultured pancreatic islets, a 50% decrease in the second phase of insulin secretion was observed when Po_2 was lowered to 10 mmHg in the perifusate (43).

In the present study, we measured the insulin content of the islet transplants to evaluate islet function in relation to tissue oxygen tension. In nondiabetic animals, the insulin content of the grafts was ~3,000 ng. Because these grafts consisted of 250 islets, this means that each islet contained on average 10–15 ng insulin. In comparison, studies of rat islets in vitro have demonstrated markedly higher insulin content levels (40–60 ng per islet) (44,45). Although β-cell death in the immediate posttransplantation period may explain some of the difference (46), low tissue oxygen tension may also contribute later on by suppressing insulin produc-

tion. In accord with previous findings (11,12,31,47,48), an even further decrease in graft insulin content was observed in the diabetic animals, compared with nondiabetic animals, in grafts implanted beneath the renal and the splenic capsules. In addition, in perfusion experiments of graft-bearing kidneys from diabetic mice, glucose-stimulated insulin secretion was markedly diminished when compared with that seen in nondiabetic control mice (49). It may be that these detrimental effects of chronic hyperglycemia on islet graft function are at least partially explained by the more pronounced hypoxia to which islet cells are exposed in the diabetic environment. Interestingly, islets implanted beneath the liver capsule had a similar insulin content irrespective of whether diabetic or nondiabetic recipients were used. At this particular implantation site, we were unable to demonstrate a decreased tissue oxygen tension in the grafts of diabetic recipients. However, whether this correlation represents a mere coincidence or a significant finding remains to be determined.

Blood glucose concentrations were slightly lower in diabetic animals with islets implanted beneath the renal capsule than in corresponding animals with islets in the two other implantation sites. However, there were no differences in insulin content among the grafts. This suggests that blood glucose concentrations may be a more sensitive method to evaluate graft function than graft insulin content. Tissue oxygen tension in the immediate neighborhood of the islet transplants varied markedly among the different implantation organs. In the spleen, a tissue oxygen tension similar to that in the pancreas was recorded, whereas the liver parenchyma had an oxygen tension similar to that recorded in the transplanted islets. Such low oxygen tension of the liver has been reported previously (50). Taken together, there was no strict correlation between the oxygen tension of the implantation organ and graft function, as evaluated by insulin content and blood glucose concentration. Therefore, the Po_2 level of the implantation organ seems to be of minor importance for the function of islet transplants.

A simple correlation between tissue oxygen tension and blood perfusion was not seen in the investigated tissues. Tissue oxygen tension in a specific location is dependent on several parameters, including the supply of oxygen to the site by blood perfusion, the diffusion capacity for oxygen, the oxygen extraction coefficient, and the cellular oxygen consumption. In addition, both the liver and the spleen have special circulatory conditions that may have impacted on the respectively low and high tissue oxygen tension recorded in these organs. In the liver, only ~20% of the blood perfusion is from the hepatic artery, whereas the remaining blood flow is venous and derived from the portal vein. The spleen has an open circulation with a huge number of erythrocytes and hemoglobin in the red pulp, which may have counteracted the influence of sluggish blood flow in this organ.

In summary, at 9–12 weeks posttransplantation we determined the tissue oxygen tension and blood perfusion in pancreatic islets transplanted to the kidney cortex, liver, or spleen. Irrespective of the implantation site, all transplanted islets had a mean Po_2 level markedly lower than that of the endogenous islets. In diabetic recipients, Po_2 levels in the transplanted islets tended to be even further decreased. Islet graft blood perfusion was similar in all islet grafts, although the three implantation organs differed markedly in their blood perfusion. Therefore, it seems that, with regard to the degree of revascularization and concomitant blood perfusion, the choice of implantation organ is of less importance than the intrinsic properties of the transplanted islets. The present study suggests that the acquired level of blood perfusion in transplanted islets at all implantation sites may be inadequate for optimal islet function. In future therapeutic approaches, techniques for optimizing islet graft revascularization should be considered.

ACKNOWLEDGMENTS

This study was supported by grants from the Swedish Medical Research Council (72X-109 and 32X-9237), the Juvenile Diabetes Foundation International, the Swedish-American Diabetes Research Program (funded by the Juvenile Diabetes Foundation and the Wallenberg Foundation), the Swedish Diabetes Association, the Swedish Society of Medicine, the Novo Nordic Fund, Svenska Barndiabetesfonden, Harald Jeanssons Stiftelse and Harald and Greta Jeanssons Stiftelse, Magnus Bergvalls Stiftelse, Familjen Ernfors Fond, and Edla och Erik Smedbergs Forskningsfond.

We gratefully acknowledge the skilled technical assistance of Astrid Nordin and Eva Törnelius.

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