Original Article

Implantation Site–Dependent Dysfunction of Transplanted Pancreatic Islets

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OBJECTIVE—Clinical islet transplantations are performed through infusion of islets via the portal vein into the liver. This study aimed at characterizing the influence of the implantation microenvironment on islet graft metabolism and function.

RESEARCH DESIGN AND METHODS—Islets were transplanted into their normal environment, i.e., the pancreas, or intraportally into the liver of mice. One month posttransplantation, the transplanted islets were retrieved and investigated for changes in function and gene expression.

RESULTS—Insulin content, glucose-stimulated insulin release, (pro)insulin biosynthesis, and glucose oxidation rate were markedly decreased in islets retrieved from the liver, both when compared with islets transplanted into the pancreas and endogenous islets. Islets transplanted into the pancreas showed normal insulin content, (pro)insulin biosynthesis, and glucose oxidation rate but increased basal insulin secretion and impaired glucose stimulation index. Gene expression data for retrieved islets showed downregulation of pancreatic and duodenal homeobox gene-1, GLUT-2, glucokinase, mitochondrial glycerol-phosphate dehydrogenase, and pyruvate carboxylase, preferentially in intraportally transplanted islets.

CONCLUSIONS—Islets transplanted into their normal microenvironment, i.e., the pancreas, display gene expression changes when compared with endogenous islets but only moderate changes in metabolic functions. In contrast, site-specific properties of the liver markedly impaired the metabolic functions of intraportally transplanted islets. ***Diabetes* 56:1544–1550, 2007**

Clinical islet transplantations have been almost exclusively performed through the intraportal route. However, despite recent advances in immunosuppressive regimens for islet allotransplantation, pancreatic islets from at least two donors are still needed to reverse hyperglycemia in type 1 diabetic patients using the intrahepatic site (1). The number of required islets is far greater than the alleged 10–20% of the total islet volume suggested to be enough to maintain normoglycemia in humans. Follow-up studies from the Edmonton group also show that there is a continuous decline in function of intraportally transplanted islets, which results in very few patients remaining insulin independent beyond 4 years posttransplantation (2). These observations, together with the occurrence of an instant blood-mediated inflammatory reaction (when exposing human islets to blood [3,4]), have in recent years seriously questioned the liver as the implantation organ of choice for pancreatic islets.

Although substantial islet cell death is likely to occur in the immediate posttransplantation period (3), dysfunction of surviving cells may also occur. This notion has been difficult to investigate, since islets implanted into the liver through the portal vein disperse within the liver parenchyma and therefore become unavailable for functional studies. However, we have recently described a method to retrieve intraportally transplanted mouse islets for functional studies and showed that such islets display severe disturbances in their glucose- and theophylline-stimulated insulin release (5). By comparison with islets implanted into their physiological microenvironment, the pancreas, the present study aimed to investigate whether this functional impairment is specific to intraportally transplanted islets or merely relates to the fact that they are transplanted per se. Our studies were extended to not only encompass glucose-stimulated insulin release but also to a number of other functional investigations and changes in the expression of genes essential for β-cell function.

RESEARCH DESIGN AND METHODS

Adult, male C57BL/6 (nu/nu) mice, weighing 25–30 g, were purchased from M&B (Ry, Denmark). YC-3.0 transgenic mice were kindly donated by Professor R.Y. Tsien at the University of California, San Diego, California, and subsequently bred at Karolinska Institutet, Stockholm, Sweden. The generation and characterization of these mice have previously been described (6,7). Briefly, transgenic YC-3.0 mice express the yellow chameleon protein 3.0 (YC-3.0) under the regulation of the β-actin and cytomegalovirus promoters. This results in the expression of enhanced yellow fluorescent protein, one part of the hybrid YC-3.0 protein, in most tissues, including all pancreatic islet cells. Glucose homeostasis, islet function, islet mass, islet vascularity, and islet blood perfusion in YC-3.0 mice have been shown to be similar to those of C57BL/6 mice (6). All animals in the present work had free access to pelleted food and tap water throughout the course of the study. The experiments were approved by the animal ethics committees of Uppsala University and Karolinska Institutet. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise indicated.

Islet isolation and culture. Pancreatic islets were isolated from adult YC-3.0 mice or C57BL/6 (nu/nu) mice by collagenase digestion (8) and cultured in groups of 150 islets for 3–4 days in 5 ml culture medium consisting of RPMI-1640 supplemented with L-glutamine, benzylpenicillin (100 units/ml;

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KRBH, Krebs-Ringer bicarbonate buffer with HEPES; LDH-A, lactate dehydrogenase-1; PDX-1, pancreatic and duodenal homeobox gene-1; TBP, TATA-box binding protein.

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Roche Diagnostics Scandinavia, Bromma, Sweden), streptomycin (0.1 mg/ml), and 10% (vol/vol) FCS. The culture medium was changed every 2nd day.

Islet transplantation. Avertin-anesthetized C57BL/6 (nu/nu) mice (0.02 ml · g^{-1} · body wt^{-1} i.p. of a 2.5% [vol/vol] solution of 10 g 97% [vol/vol] 2,2,2-tribromo-ethanol in 10 ml 2-methyl-2-butanol [Kemila, Stockholm, Sweden]) were used as recipients. After culture, 200 YC-3.0 islets were packed in a butterfly needle (25 gauge) and injected either into the portal vein, as previously described (9), or directly into the splenic part of the pancreas of the recipients. In the latter case, the injection needle was slowly retracted during injection to spread the islets and avoid clustering of transplanted tissue within the pancreas.

Retrieval of intraportally transplanted islets. At 1 month posttransplantation, the intraportally transplanted animals were anesthetized with avertin and the islets retrieved as previously described (5). The retrieved islets were cultured 1–4 days before functional analysis.

Retrieval of intrapancreatically transplanted islets. At 1 month posttransplantation, intrapancreatically transplanted islets were retrieved and isolated using a similar collagenase method as used for isolation of endogenous islets. Briefly, the pancreas was removed, washed in HBSS (Hanks' balanced salt solution), cut into pieces, and transferred to a vial containing collagenase (3.125 mg/ml) dissolved in 8 ml HBSS. The vial was shaken at 37°C for ~20 min until the tissue had disintegrated, leaving free islets. The pancreatic digest was washed before the fluorescent YC-3.0 islets were handpicked and separated from the endogenous islets under a fluorescence microscope. The retrieved YC-3.0 islets, as well as endogenous islets from C57BL/6 (nu/nu) mice, were cultured for 1–4 days.

Immunohistochemistry. Endogenous islets and islets retrieved following transplantation were processed for immunohistochemical stainings. The islets were fixed in 4% (vol/vol) paraformaldehyde and embedded in 5% (wt/vol) agarose (SeaKem LE Agarose; Cambrex Bio Science Rockland, Rockland, ME). Thereafter, the agarose-embedded islets were placed in 4% (vol/vol) neutral buffered formalin and paraffin embedded. The tissue was sectioned (5 μm), mounted on glass slides, and stained with antibodies against insulin (ICN Biomedicals, Aurora, OH) and glucagon (Fitzgerald, Concord, MA) as previously described (10). These sections were then examined using a point-counting method (11), where the number of intersections overlapping insulin- and glucagon-positive cells, respectively, was counted in a light microscope (12). In each animal, 260 ± 18 points were investigated. The mean percentages of insulin- and glucagon-positive cells of islets from each animal were calculated and considered as one observation in the further statistical analysis.

Glucose-stimulated insulin release. After 3–4 days of culture, groups of 10 endogenous or retrieved YC-3.0 islets were transferred to vials containing Krebs-Ringer bicarbonate buffer supplemented with 10 mmol/l HEPES and 2 mg/ml BSA (KRBH) (ICN Biomedicals). The KRBH buffer contained 1.67 mmol/l D-glucose during the 1st h of incubation at 37°C (O_2:CO_2, 95:5). Thereafter, medium was replaced by KRBH supplemented with 16.7 mmol/l glucose and the islets incubated for a 2nd h. Following the retrieval of the medium, the islets were harvested and homogenized by sonication in 200 μl redistilled water. Aliquots of the aqueous homogenate were used for DNA measurements by fluorophotometry (PicoGreen dsDNA Quantitation Kit catalogue no. P-7589; Molecular Probes, Eugene, OR). A fraction of the homogenate was mixed with acid ethanol (0.18 mol/l HCl in 95% [vol/vol] ethanol), from which insulin was extracted overnight. Insulin contents in incubation media and homogenates were determined by a mouse insulin enzyme-linked immunosorbent assay kit (Mercodia, Uppsala, Sweden).

To investigate whether implanted YC-3.0 islets in the pancreas exerted a negative influence on the endogenous islets of the recipient athymic mice, we also determined the glucose-stimulated insulin release capacity from such endogenous islets and compared it with that from endogenous islets obtained from other athymic mice not subjected to islet transplantation.

Glucose oxidation. Islet glucose oxidation rates were determined after 3–4 days of islet culture for groups of 10 islets according to a previously described method (13). All glucose oxidation rate values were normalized for and expressed per microgram of DNA, since islet sizes tended to differ, although this did not reach statistical significance (see RESULTS).

(Pro)insulin biosynthesis. Endogenous islets and islets retrieved from the liver or pancreas were cultured overnight before determining the (pro)insulin and total protein biosynthesis for groups of 10 islets as previously described (14).

Gene expression analysis. After overnight culture, isolation of total RNA was performed with RNeasy Mini Kit (Qiagen, Hilden, Germany), and all of the samples were treated with RNase-free DNase (Qiagen). The RNA quality was analyzed with the Agilent Bioanalyzer 2100 using RNA Pico Chips (Agilent Technologies, Waldbronn, Germany). Conversion of RNA to cDNA was performed with a reverse-transcription system (Promega SDS Biosciences, Falkenberg, Sweden) using Oligo $(dT)_{15}$ primers. The LightCycler System (Roche Diagnostics, Mannheim, Germany) and detection with SYBR Green was used to amplify and analyze generated cDNA. The primers used (Tebu-Bio, Roskilde, Denmark) were GLUT-2 (Genebank accession no. NM_031197, catalog no. PPM04167A), glucokinase (Gck) (NM_010292, PPM05095A), mitochondrial glycerol-phosphate dehydrogenase (mGPDH) (NM_010274, PPM03293A), and pyruvate carboxylase (Pcx) (NM_008797, PPM05018A). Additional primers used were as follows: TATA-box binding protein (TBP) (Genebank accession no. D01034; Cybergene, Huddinge, Sweden), 5′-ACC CTT CAC CAA TGA CTC CTA TG-3′, 5′-ATG ATG ACT GCA GCA AAT CGC-3′; uncoupling protein 2 (AF096288; MWG, Ebergsberg, Germany), 5′-AGC CTA CAA GAC CAT TGC ACG-3′, 5′-CAG AAG TGA AGT GGC AAG GGA-3′; lactate dehydrogenase-1 (LDH-A) (NM_010699; MWG), 5′-GGT TGC AAT CTG GAT TCA GCG-3′, 5′-TCA GTG CCC AGT TCT GGG TTA-3′; and pancreatic and duodenal homeobox gene-1 (PDX-1) (NM_008814; TIB MOLBIOL, Berlin, Germany), 5′-GGT GCC AGA GTT CAG CGC TA-3′, 5′-TTG TTT TCC TCG GGT TCC GC-3′.

PCR amplifications were performed in a total volume of 10 μl, containing 1 μl cDNA, 0.5 μmol/l of each primer, 4 μl SYBR Green JumpStart Taq ReadyMix, and RNase-free water added to the final volume. Each RT-PCR run started with denaturation at 94°C for 10 s. Cycling parameters were 94°C for 10 s, 57°C for 12 s, and 72°C for 8 s. The results are presented as threshold cycle values (C_T values), i.e., the estimated amplification cycle number when the fluorescence exceeds a specified threshold value. The C_T values were used to calculate the amount of PCR product in comparison with TBP as a "house-keeping gene" by subtracting the C_T value for TBP from the C_T value for the gene studied (ΔC_T). Relative mRNA expression was calculated as $2^{-\Delta Ct}$.

Statistical analysis. All values are given as means ± SEM. Multiple comparisons for normally distributed data were performed using ANOVA and the Bonferroni post hoc test, whereas nonparametric values were compared using nonparametric ANOVA and Dunn's test. For all comparisons, a P value <0.05 was considered statistically significant.

RESULTS

Identification and cell composition of transplanted islets retrieved from liver and pancreas. The retrieved islets could easily be distinguished from the surrounding tissues in a fluorescence microscope (liver tissue, exocrine pancreas, and endogenous C57BL/6 [nu/nu] islets) by the enhanced yellow fluorescent protein fluorescence selectively expressed by the YC-3.0 islets (Fig. 1*A*–*F*). The identity of the retrieved tissue as islets was further confirmed by staining with insulin antibodies in sections prepared for light microscopy (Fig. 1*H*–*I*). There was no difference in the percentage of insulin-positive cells between control YC-3.0 islets and islets retrieved from the pancreas or liver (76.0 ± 3.0, 71.2 ± 1.3, and 70.4 ± 4.2%, respectively; $n = 4$ animals in each group). Transplanted islets retrieved from the pancreas also had a similar percentage of glucagon-positive cells as control islets, whereas the percentage of glucagon-positive cells in islets retrieved from the liver tended to be lower (21.4 ± 0.6, 22.3 ± 1.7, and 7.4 ± 0.7% for endogenous islets and islets retrieved from the pancreas or liver, respectively; $P < 0.05$ for intrahepatic islets vs. intrapancreatic islets).

Size range and retrieval rate of endogenous and transplanted islets. The DNA content of control islets and islets retrieved from the liver or pancreas did not differ statistically (0.208 ± 0.0188 [$n = 5$], 0.407 ± 0.0923 [$n = 7$], and 0.325 ± 0.0283 [$n = 6$] μg/10 islets, respectively; $P = 0.143$). The retrieval rate of transplanted islets reached 75% but occasionally only 20–30%.

Glucose-stimulated insulin release. Nontransplanted control YC-3.0 islets responded to the medium with a 30-fold increase in insulin release on high-glucose stimulation (16.7 mmol/l) (Fig. 2*A*). In contrast, retrieved intraportally transplanted islets did not increase insulin release at all on exposure to high glucose. Islets transplanted to the pancreas had an increased insulin secretion already at exposure to low glucose (1.67 mmol/l) compared with

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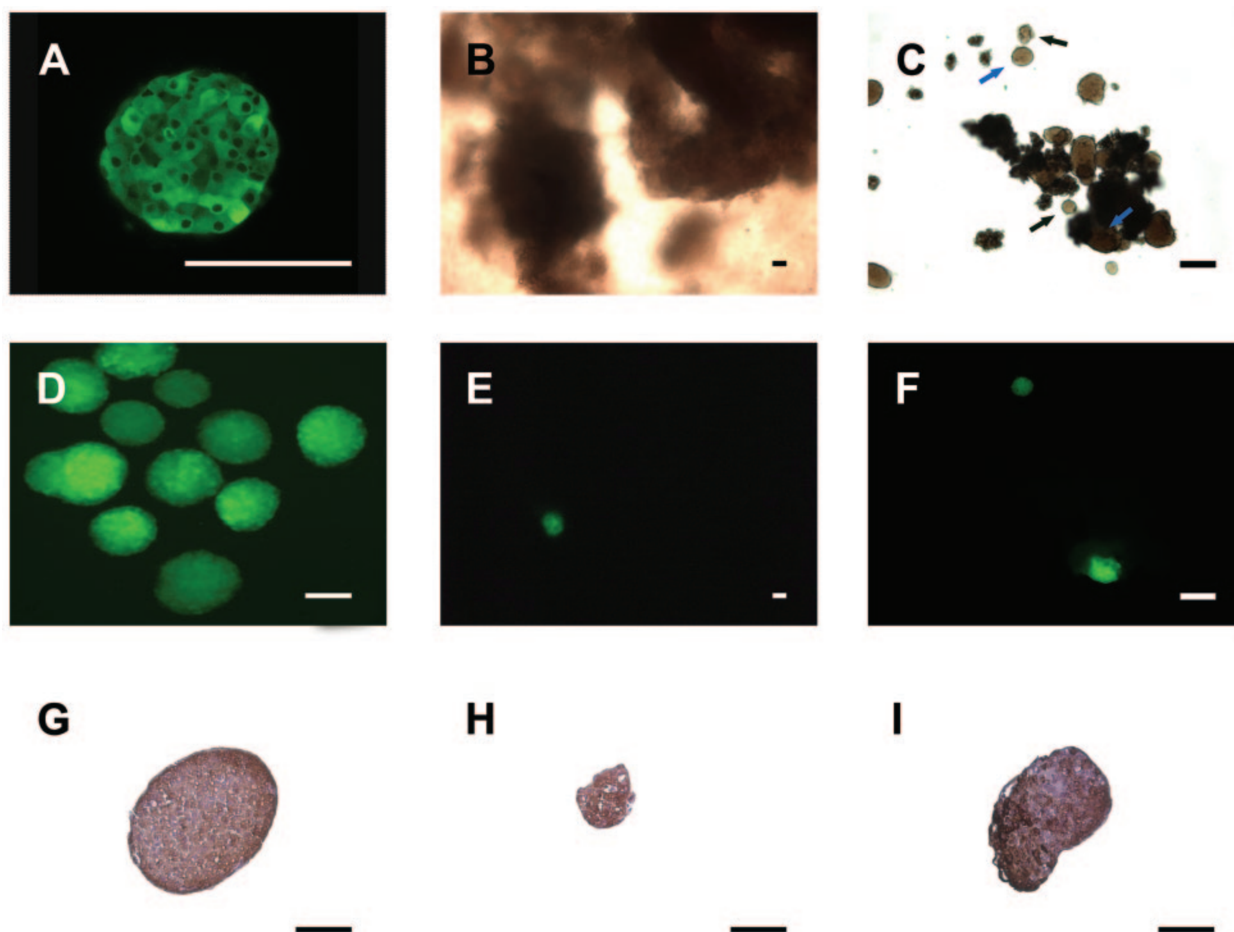

FIG. 1. Confocal image of an YC-3.0 islet showing the ubiquitous expression of the enhanced yellow fluorescent protein in islet cells (*A*). Light microscopic images of digests of graft-bearing liver (*B*) and pancreas (*C*) following collagenase treatment 1 month after transplantation. Note in *C* that the nonfluorescent endogenous C57BL/6 (nu/nu) islets (black arrows) are inseparable from the transplanted YC-3.0 islets (blue arrows) in white light. Endogenous islets obtained from a YC-3.0 mouse (*D*), and transplanted YC-3.0 islets retrieved from the liver (*E*) or pancreas (*F*), instead visualized with fluorescence. Note that the images in *E* and *F* are the same as those photographed in conventional white light in *B* and *C*, respectively. Insulin staining of an endogenous YC-3.0 islet (*G*) and fluorescent tissue retrieved from the liver (*H*) or pancreas (*I*). The scale bars correspond to 100 μm.

control islets. When stimulated with high glucose, their insulin release was further increased but did not reach the same level as that for control islets.

Endogenous islets obtained from athymic mice with an intrapancreatic graft responded similarly to control islets from athymic mice with insulin release in response to low and high glucose exposure (Fig. 2*B*).

Insulin content. Islets transplanted to the pancreas had similar insulin content as control YC-3.0 islets. In contrast, intraportally transplanted islets had markedly lower insulin content compared both with control YC-3.0 and with intrapancreatically transplanted islets (Fig. 3).

Glucose oxidation. The glucose oxidation rate in transplanted islets retrieved from the pancreas was similar to that in control YC-3.0 islets. On the contrary, islets retrieved from the liver had a markedly lower glucose oxidation rate than that both in control YC-3.0 and in intrapancreatically transplanted islets (Fig. 4).

(Pro)insulin biosynthesis. Transplanted islets retrieved from the liver had a markedly decreased (pro)insulin biosynthesis compared both with control YC-3.0 and intrapancreatically transplanted islets (Fig. 5*A*). The (pro)insulin biosynthesis in the intrapancreatically transplanted islets varied quite extensively between samples, but as a mean it was similar to that in control islets. Total protein biosynthesis was similar in control islets and islets transplanted to the liver, whereas it was increased two- to threefold in intrapancreatically transplanted islets (data not shown).

When (pro)insulin biosynthesis was expressed as a fraction of total protein biosynthesis, the values were similar for control and intrapancreatically transplanted islets. However, intraportally implanted islets also had a markedly lower value in this context (Fig. 5*B*).

Gene expression analysis. The mRNA levels of PDX-1 were markedly decreased in islets transplanted to the liver and pancreas compared with control YC-3.0 islets (Fig. 6*A*). However, no differences in PDX-1 expression were discerned between islets transplanted to the liver or pancreas. Likewise, the mRNA levels of GLUT-2 were similarly decreased at both implantation sites after transplantation compared with control YC-3.0 islets (Fig. 6*B*). Islets retrieved from the liver had lower Gck mRNA levels than control YC-3.0 islets, whereas this was not the case for intrapancreatically transplanted islets (Fig. 6*C*). The key mitochondrial enzymes Pcx and mGPDH were also markedly downregulated in intraportally transplanted islets, whereas only Pcx was decreased in intrapancreatically transplanted islets (Fig. 6*D–E*). The mRNA levels of mGPDH in intrapancreatically transplanted islets were

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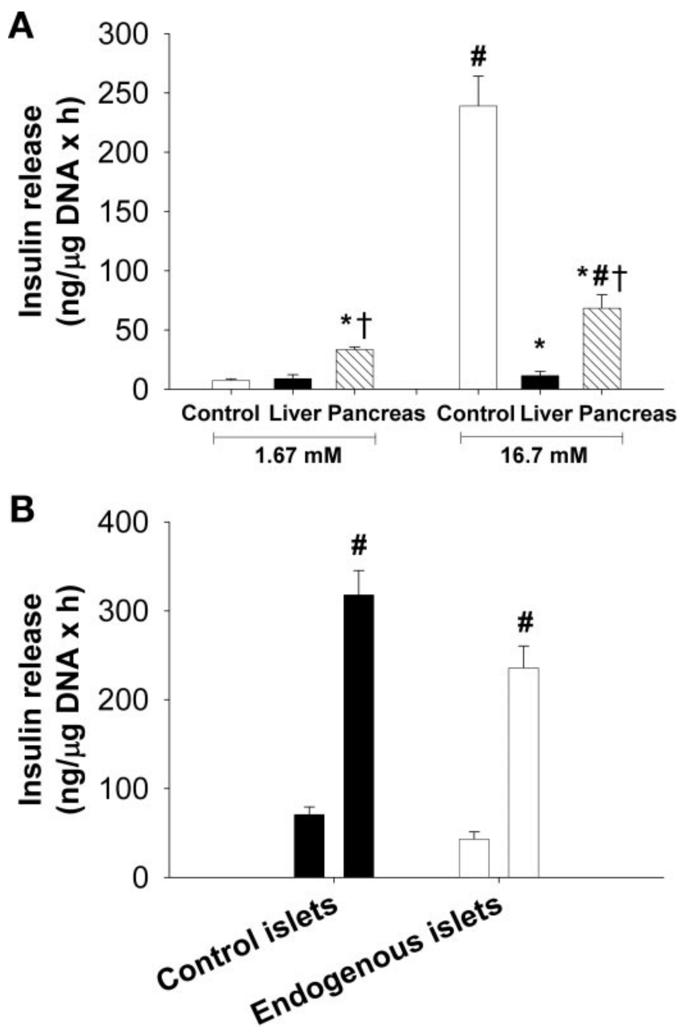

FIG. 2. Glucose-stimulated insulin release in control YC-3.0 islets (□) and in islets retrieved from the liver (■) or pancreas (▧). Data are means ± SEM for five to nine animals in each group. *B*: Glucose-stimulated insulin release in control islets of athymic mice (■) and in endogenous islets obtained from athymic mice with an intrapancreatic graft (□). *$P < 0.05$ compared with control islets, †$P < 0.05$ compared with islets retrieved from the liver, and #$P < 0.05$ compared with corresponding values obtained at low glucose stimulation.

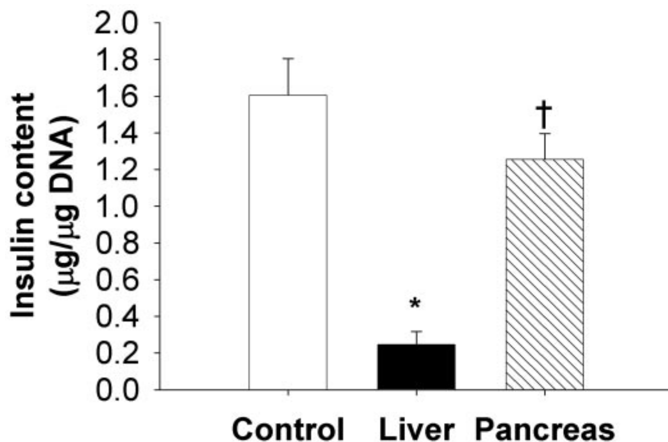

FIG. 3. Insulin content in control YC-3.0 islets (□) and in islets retrieved from the liver (■) or pancreas (▧). Data are means ± SEM for five to nine animals in each group. *$P < 0.05$ compared with control islets, whereas †$P < 0.05$ compared with islets retrieved from the liver.

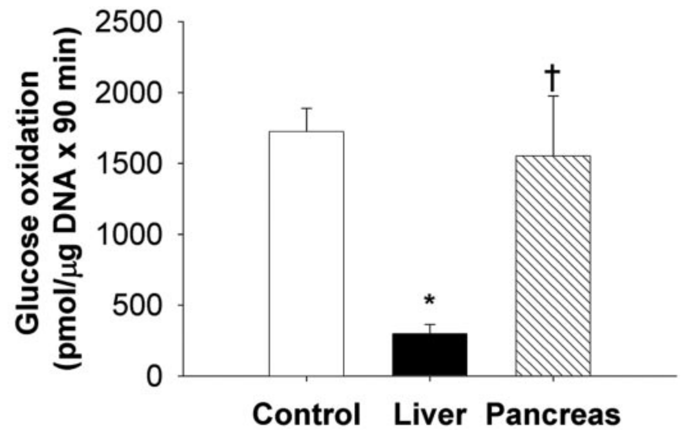

FIG. 4. Glucose oxidation rates in control YC-3.0 islets (□) and in islets retrieved from the liver (■) or pancreas (▧). Data are means ± SEM for five to nine animals in each group. *$P < 0.05$ compared with control islets, whereas †$P < 0.05$ compared with islets retrieved from the liver.

higher than in intraportally transplanted islets. Interestingly, there was a substantial increase in the expression of LDH-A in intrapancreatically transplanted islets, while there was only a tendency toward elevated LDH-A levels in intraportally transplanted islets (Fig. 6*F*). No differences in the mRNA levels of uncoupling protein 2 were observed in the different groups (Fig. 6*G*).

DISCUSSION

The present study demonstrates that transplanted pancreatic islets retrieved from the liver microenvironment display severe functional disturbances. A perturbed glucose-stimulated insulin release correlated with defects in glucose oxidation, (pro)insulin biosynthesis, and decreased insulin content. In comparison, transplanted islets retrieved from the pancreas showed similar glucose oxidation rates, (pro)insulin biosynthesis, and insulin content as nontransplanted control islets. Furthermore, intrapancreatically transplanted islets secreted more insulin in response to glucose than intraportally transplanted islets, though less than in control islets. Taken together, our results strongly suggest that the function of transplanted islets indeed is influenced by their implantation microenvironment.

The observed functional impairments are not the only changes that have been reported for intraportally transplanted islets. Also, a defective glucagon response to hypoglycemia (15,16) and a decrease in the number of α-cells (12) seem to preferentially occur at this site. In fact, experimental studies in rodents have indicated a lack of long-term function of intraportally transplanted islets compared with islets implanted to other sites (17). In the present study, the loss of function in intraportally transplanted islets was associated with changes in the expression of several genes essential for normal β-cell function. Thus, markedly decreased islet gene expression was observed not only for the β-cell differentiation marker PDX-1 but also for key enzymes in glucose transport and metabolism in the β-cells, such as GLUT-2, Gck, Pcx, and mGPDH. Confirming our previous results in rats (12), there was no obvious decrease in β-cell number of intraportally transplanted islets as evidenced by the insulin stainings of retrieved islets. Instead, the present results suggest a clear shift in β-cell phenotype, similar to that seen in response to

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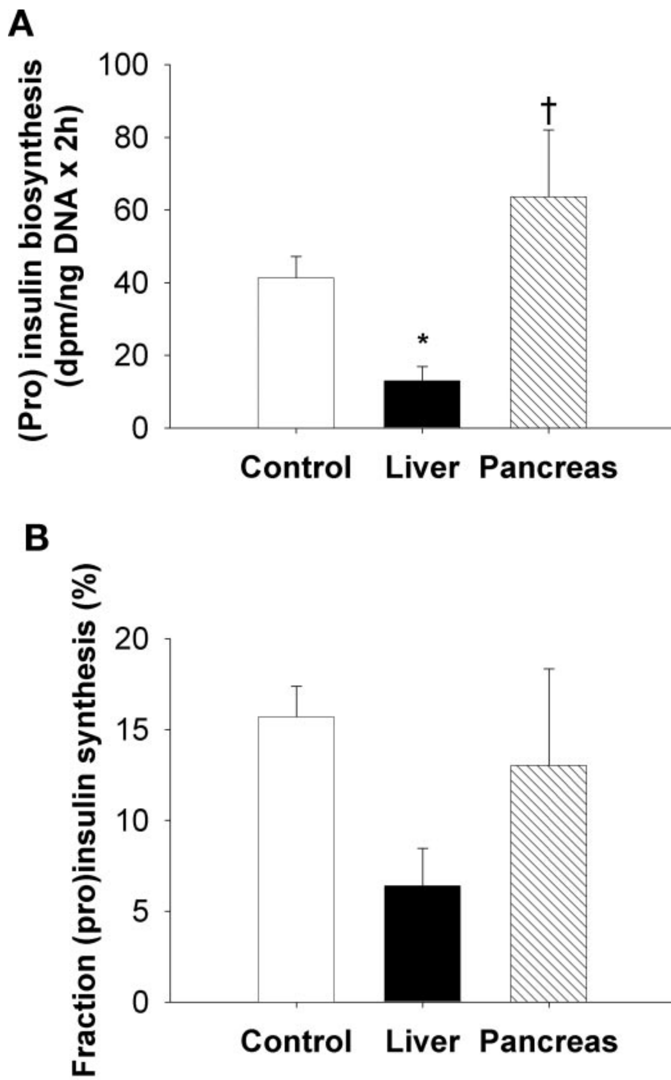

FIG. 5. (Pro)insulin biosynthesis (*A*) and fraction (pro)insulin of total protein biosynthesis (*B*) in control YC-3.0 islets (□) and in islets retrieved from the liver (■) or pancreas (▧). Data are means ± SEM for five to six animals in each group. *$P < 0.05$ compared with control islets, whereas †$P < 0.05$ compared with islets retrieved from the liver.

chronic hyperglycemia (18). All observed gene expression changes could cause impairments in glucose-induced ATP synthesis and insulin secretion, thereby providing a molecular basis for the β-cell dysfunction.

The intrahepatic microenvironment can be expected to provide several site-specific challenges for implanted islets, which may contribute to the observed gene expression changes and functional impairments. Intraportally implanted islets are chronically exposed to high levels of glucose absorbed from the intestine and produced by the hepatocytes. Immediately after transplantation, the islet cells are also avascular and most likely hypoxic, since the liver parenchyma has an oxygen tension of only 5–10 mmHg, which is markedly lower than that seen in most other tissues, including the pancreas (19,20). Moreover, although intraportally transplanted islets later seem to become revascularized from the hepatic artery (12,21), the resulting vascular density is much lower than in endogenous islets (10) and islets implanted in the pancreas (C. Kampf, G.M., J.L., D.N., M.L., P.-O.B., P.-O.C., unpublished observations). In this aspect, a decreased oxygen and nutrient transport, perturbed disposal of secreted hormones, and loss of endothelial paracrine interactions may all potentially change the islet phenotype.

The endocrine function of retrieved intraportally transplanted islets seemed even more impaired than expected based on previous results from perfusion of graft-bearing livers in rat (12,22,23). We have previously thoroughly evaluated our technique to retrieve intraportally transplanted islets and found that the procedure in itself is unlikely to damage the islet cells (5). In some cases, we also retrieved islets from graft-bearing livers without prior intravascular collagenase perfusion and instead only disintegrated the liver tissue by exogenous treatment during incubation at 37°C in a shaking bath. Lower numbers of islets were retrieved, but they displayed similar changes as islets retrieved by the perfusion technique (data not shown). The fact that our retrieved islets still had a poor performance after several days in culture further supports the view that the functional defects are inherent to the islets themselves and not dependent on the retrieval procedure. It is possible that differences in results between perfused graft-bearing rat livers and the retrieved

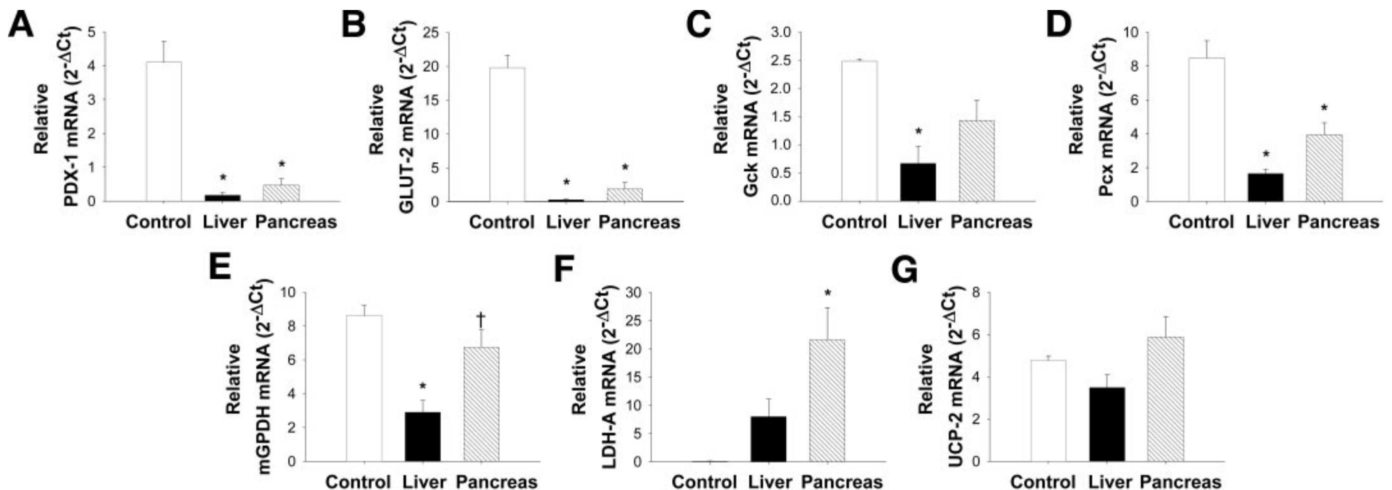

FIG. 6. Gene expression for PDX-1 (*A*), GLUT-2 (*B*), Gck (*C*), Pcx (*D*), mGPDH (*E*), LDH-A (*F*), and uncoupling protein-2 (*G*) in control YC-3.0 islets (white bars) and in islets retrieved from the liver (black bars) or pancreas (hatched bars). Data are means ± SEM for four to six animals in each group. *$P < 0.05$ compared with control islets, whereas †$P < 0.05$ when compared with islets retrieved from the liver.

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mouse islets in the present study merely represent species differences in the susceptibility to liver-induced islet dysfunction. However, another more likely explanation is that small islet fragments and single islet cells become better engrafted than intact islets and, therefore, constitute the major functional population of β-cells in the liver. Islet fragments are, because of their small size compared with whole islets, difficult to reliably identify after reisolation. Only ~65% of intraportally transplanted islets seem to remain in the liver as intact islets (24), and, of these, up to 75% could in some cases be retrieved. Since the DNA content of our islets retrieved from the liver tended to be higher than that of control islets and islets retrieved from the pancreas, we may preferentially have detected the larger islets in the digest of the liver. However, although larger functional variations were observed in islets retrieved from the liver than in control islets, they nevertheless consistently had impaired function.

Despite being implanted into their normal physiological microenvironment, intrapancreatically transplanted islets also displayed some functional and gene expression changes. A characteristic finding for the retrieved intrapancreatically transplanted islets was their higher insulin release compared with control islets at low glucose concentrations. This observation is unlikely to solely reflect leakage from damaged β-cells induced by reisolation, since the islets were cultured for 3–4 days before the insulin release experiments to remove damaged cells. Instead, this may be an inherent change in islets implanted into the pancreas as suggested by the finding that animals receiving large numbers of intrapancreatically transplanted islets are slightly hypoglycemic (25). Interestingly, intrapancreatically transplanted islets demonstrated a high expression of the LDH-A gene, which is normally expressed in low levels in β-cells. This occurred despite the presence of a similar fraction of β-cells in the intrapancreatic grafts as in control islets. We were not able to retrieve and investigate single islets from the renal subcapsular site, since islets implanted to this site adhered tightly to each other and surrounding connective tissue stroma. However, we have previously observed increased lactate formation and increased lactate-to-pyruvate ratios for clusters of islets transplanted beneath the renal capsule (26,27). Also, the intraportally transplanted islets in the present study tended to have increased expression of LDH-A. A low expression level of LDH-A has been claimed to be important to direct carbon molecules into oxidative phosphorylation and ATP production (28). However, when LDH-A were overexpressed in INS-1 cells, lactate, even at low concentrations and in the absence of glucose, caused insulin secretion (29). This finding provides a potential explanation for the increased insulin secretion observed from retrieved intrapancreatically transplanted islets at low glucose conditions in the present work. Interestingly, exercise has recently been shown to induce hypoglycemia in rats bearing islet transplants in different sites (30). It can be speculated that this response reflects insulin secretion from the transplanted β-cells when exposed to increased circulating lactate concentrations. Besides increased LDH-A expression, intrapancreatically transplanted islets showed decreased expression of PDX-1, GLUT-2, and Pcx. All of these changes could cause the decreased glucose-stimulated insulin release also observed in the intrapancreatically transplanted islets.

In our studies, we chose to use normoglycemic rather than diabetic recipients to avoid differences in blood glucose concentrations between recipients, which may have affected islet transplant function. We have previously, in several studies, observed that the function and engraftment of transplants are similar in normoglycemic and successfully cured diabetic recipients (5,20,31,32). In the present study, we instead evaluated whether islets transplanted to the pancreas affected the function of endogenous islets. This was not the case, since endogenous islets obtained from mice bearing an intrapancreatic graft responded similarly to control islets regarding insulin release in response to low and high glucose exposure.

We conclude that site-specific properties of the liver markedly perturb the metabolic functions of intraportally transplanted islets. Although changes in gene expression and function also occur in intrapancreatically transplanted islets, these disturbances were not as profound as those observed for islets implanted in the liver. This study provides further evidence suggesting that the liver may be an inappropriate choice of organ for implantation of islets in the clinical situation and that other sites should be considered.

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