

Metabolic Mechanisms of Failure of Intraportally Transplanted Pancreatic β -Cells in Rats

Role of Lipotoxicity and Prevention by Leptin

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The objective of this study was to determine whether the late failure of β -cells in islets transplanted via the portal vein is caused by excess insulin-stimulated lipogenesis and lipotoxicity and, if so, whether the damage can be prevented by reducing lipogenesis surrounding the islets. Based on the premise that high portal vein levels of nutrients and incretins would stimulate hyperinsulinemia, thereby inducing intense lipogenesis in nearby hepatocytes, normal islets were transplanted into livers of syngeneic streptozotocin-induced diabetic recipients. Hydrolysis of the surrounding fat would flood the islet grafts with fatty acids that could damage and destroy the β -cells. Reducing lipogenesis by leptin or caloric restriction should prevent or reduce the destruction. After a rise after transplantation, insulin levels gradually declined and hyperglycemia increased. Four weeks after transplantation mRNA of the lipogenic transcription factor, sterol regulatory element-binding protein-1c (SREBP-1c) and its lipogenic target enzymes were elevated in livers of these recipients, as was triacylglycerol content. Positive oil red O staining for lipids and immunostaining for SREBP-1 were observed in hepatocytes surrounding islets with damaged β -cells. Leptin-induced lipopenia prevented and caloric restriction reduced steatosis, hyperglycemia, and apoptotic β -cell destruction. Excessive SREBP-1c-mediated lipogenesis, induced in hepatocytes by insulin hypersecretion, is followed by β -cell destruction in the grafts and reappearance of diabetes. Graft failure is prevented by blocking lipogenesis. The results suggest that strict antilipogenic intervention might improve outcomes after human islet transplantation. *Diabetes* 56:2295–2301, 2007

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Received for publication 3 April 2007 and accepted in revised form 26 May 2007.

Published ahead of print at <http://diabetes.diabetesjournals.org> on 11 June 2007. DOI: 10.2337/db07-0460.

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ACC, acetyl CoA carboxylase; FAS, fatty acid synthetase; SCD-1, stearoyl CoA desaturase-1; SREBP-1, sterol regulatory element-binding protein-1; TUNEL, transferase-mediated dUTP nick-end labeling.

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Transplantation of the pancreas or of isolated islets is presently the only curative therapy for type 1 diabetes. In contrast to pancreas transplantation, islet transplantation is a minimally invasive, relatively benign procedure that can be done on an outpatient basis. Unfortunately, early results with islet transplantation were disappointing, with few centers reporting sustainable insulin independence (1). This high rate of late transplant failure has been attributed to immunorejection, toxicity of immunosuppression agents, and aberrant blood delivery in the recanalized intrahepatic islet grafts (2).

Since then, introduction of a new immunosuppressive regimen, known as the Edmonton protocol, has dramatically improved the results (3). On this corticosteroid-free immunosuppressive program, consisting of sirolimus, low-dose tacrolimus, and anti-interleukin-2 receptor antibody, ~70% of transplant recipients become insulin independent during a 5-year follow-up, but only ~13% maintain insulin independence for 2 years (1), despite persistence of C-peptide levels (2). The evidence that not all β -cells are destroyed, together with the salutary effect of elimination of glucocorticoids from the treatment program, raised the possibility that slow, incomplete loss of islets was not the result of a destructive immunological process. Rather, the slow, incomplete β -cell dysfunction was more reminiscent of type 2 diabetes than rejection.

A well-described cause of slow, incomplete β -cell dysfunction and death in rodents is known as "lipotoxicity." This process was first identified in obese Zucker diabetic fatty (ZDF) rats in which a progressive overaccumulation of lipids in native islets leads to β -cell destruction and type 2 diabetes (4). The consequences of lipid overaccumulation in vivo were duplicated by culturing normal islets in long-chain fatty acids at concentrations comparable with those observed in ZDF rats (5). Moreover, in vivo destruction of native islets of prediabetic ZDF rats could be prevented by measures that reduced lipid accumulation (6,7).

There is a plausible theoretical basis for considering lipotoxic destruction as a cause of failure of human islet transplants. Chronic exposure of normal islets transplanted to the liver would expose them to high portal vein levels of nutrients and gut hormones; the resulting hypersecretion of undiluted insulin into surrounding hepatocytes would elicit a powerful lipogenic response, overloading the

nearby hepatocytes with triacylglycerol. Islets would be chronically exposed to both a uniquely high lipid environment and a high glucose environment. This combination would result in glucolipotoxicity. This idea is supported by liver biopsy evidence (8–10) and by a report that fatty liver diagnosed by magnetic resonance imaging (11) occurs in 20% of subjects in association with graft dysfunction (12). The potential pathogenic consequences of the lipid excess are suggested by the fact that exposure of isolated human islets to fatty acids damages β -cells and directly or indirectly results in apoptosis (13).

Because late failure of islet transplants is a serious limitation in a procedure that might otherwise benefit larger numbers of patients with type 1 diabetes, we have attempted to determine whether the overaccumulation of lipids in the hepatocytes surrounding the islets creates a lipotoxic environment that damages β -cells in islet grafts and, if so, whether the damage can be prevented by lowering the lipid content in the liver.

RESEARCH DESIGN AND METHODS

Lean, wild-type ZDF (+/+) rats were housed in individual cages with constant temperature and 12 h of light alternating with 12 h of darkness. All rats were fed Teklad 6% mouse/rat diet (Teklad, Madison, WI) and had free access to water. Diabetes was induced at 10–12 weeks of age by intravenously streptozotocin (80 mg/kg body wt). Within 7 days all rats were profoundly hypoinsulinemic, with nonfasting hyperglycemia above 400 mg/dl, polyuria, and weight loss of 36–40 g. At that point, a suboptimal dose of ~1,500 pancreatic islets, isolated as described previously (4) from related wild-type lean ZDF(+/+) rats, was injected over 5 min into the portal vein. Three groups of six recipients were studied. One group received no treatment or diet restriction (ad lib-fed group), a second group was diet-restricted by 50%, and a third group received intravenously 1×10^{12} plaque-forming units of adenovirus containing the leptin cDNA. In addition, a group of untransplanted streptozotocin-induced diabetic rats were compared with a group of normoglycemic high-dose islet recipients.

Food intake was measured daily; body weight and blood glucose were measured weekly. Four weeks later, animals were killed. Plasma glucose was measured by a PGO glucose kit (Sigma, St. Louis, MO). Plasma leptin and insulin were measured by Linco rat leptin and sensitive rat insulin kits (Linco Research, St. Charles, MO). The protocol was approved by the Institutional Animal Care and Research Advisory Committee of the University of Texas Southwestern Medical Center and the Institutional Animal Care and Use Committee at the Veterans Administration Medical Center.

Morphology. Livers were perfused with 10% formaldehyde and frozen in liquid nitrogen for oil red O staining or fixed in Bouin's solution for insulin immunocytochemistry and collagen staining. These were carried out, as previously described (14), at the University of Geneva with a monoclonal insulin antibody provided by M.J. Storch. Morphometric analysis for insulin cells was performed in Dallas, Texas, using Image-J image analysis software and particle analysis macro (Scion, Frederick, MD). The area of insulin staining in three sections of liver from three animals, relative to total sectional area examined, was quantified by monochromatic thresholding. Morphometric analysis of fibrosis in islet transplants was performed in Geneva on paraffin sections using Goldner trichrome staining. Pictures were taken with an Axiophot microscope, objective $\times 40$. Volume density was calculated by point counting.

Transferase-mediated dUTP nick-end labeling (TUNEL) staining of livers perfused with 4% paraformaldehyde was performed in Dallas, using the Promega Dead-End Fluorometric kit (Promega, Madison, WI). Morphometric analysis of TUNEL staining was performed by counting positive granules in three sections from the liver of three rats in the different groups.

Sterol regulatory element-binding protein-1 (SREBP-1) immunostaining was performed in Dallas. Cryostat sections were incubated overnight at 4°C with 1:50 dilution of a polyclonal anti-SREBP-1 (amino acids 31–175) antibody, provided by Michael S. Brown and Joseph L. Goldstein. The sections were washed with PBS and treated with 1:20 dilution of biotinylated goat anti-rabbit and 1:500 peroxidase streptavidin for 30 min. Dako diaminobenzidine chromogen (Dako Cytomation, Carpinteria, CA) was applied for 5 min and counterstained with hematoxylin and eosin.

Liver triacylglycerol content. Hepatic lipids were extracted by the method of Folch (15) and dried under N_2 gas as described previously (4). Triacylglycerol content was assayed using the GPO-Trinder Sigma kit.

SREBP-1 immunoblotting. Immunoblotting for SREBP-1 in liver fractions was carried out as described previously (16,17).

Real-time quantitative PCR. Total RNA was extracted from liver by TRIzol isolation (Life Technologies, Rockville, MD). All PCR reactions were performed in triplicate using the following primers. SREBP-1c: forward primer, GGAGCCATGGATTGCACATT, and reverse primer, AGGCCAGGGAAGTCAC TGTCT (accession no. AF286469); SREBP-2: forward primer, CTGCAGATC CCGCAGTACAG, and reverse primer, GGTGGATGAGGGAGAGAAGGT (accession no. NM_001033694); stearoyl CoA desaturase-1 (SCD-1): forward primer, AAGATATCCACGACCCAGCTA, and reverse primer, TGCAGCAG GGCCATGAG (accession no. NM_139192); and fatty acid synthetase (FAS): forward primer, GACCCGACTCCAAGTTATTCCGA, and reverse primer, CGT CAAGCGGGAGACAGACT (accession no. NM_017332). The forward primer for the invariant control rat cyclophilin was CGTGGGCTCCGTTGTCTT, and the reverse primer was TGACTTTAGGTCCCTTCTTCTATCG (accession no. NM_022536).

Statistical analysis. All results are expressed as mean \pm SE. The statistical significance of differences in mean values was assessed by Student's *t* test for two groups.

RESULTS

Rationale for β -cell lipotoxicity in failure of intra-portal islet transplants. To create an insulin-deficient diabetic recipient model equivalent to human recipients of islet transplants, we induced severe diabetes with streptozotocin. One week later, we injected ~1,500 islets, isolated from normal, closely related donors, into the portal vein of six recipients. We hypothesized that in this milieu, the β -cells of their grafts would be chronically hyperstimulated by the high portal venous levels of nutrients and incretins perfusing the intrahepatic islet transplants. High, undiluted levels of insulin would flood nearby hepatocytes with insulin, eliciting intense lipogenesis, while the residual hyperglycemia of the streptozotocin-induced diabetes would assure ample substrate for glucose-derived lipid synthesis. Hepatocytes closest to islet transplants would produce the highest abundance of VLDL. The islet lipoprotein lipase (18) would hydrolyze triacylglycerol, creating local excess fatty acids that could diffuse into islet cells by rapid “flip-flopping” across their plasma membranes (19). If the lipotoxicity concept is correct, this lipid excess should injure and kill β -cells through one or more of the pathways that lead to apoptosis (20–24), and hyperglycemia should worsen. Furthermore, if lipid excess is the primary factor, it should be preventable by reducing the lipid excess surrounding the islets. The following experiments were designed to test this hypothesis.

Gradual functional loss of β -cells in islets transplanted to the liver of syngeneic diabetic recipients. Administration of 80 mg/kg streptozotocin to six normal rats induced severe diabetes with polydipsia, polyphagia, and weight loss. Blood glucose averaged 449 ± 32 mg/dl. One week later, after losing 36–40 g body wt, the rats received an intraportal infusion of ~1,500 islets, after which their mean insulin level rose to 0.89 ± 0.05 ng/ml and blood glucose declined to 298 ± 23 mg/dl. Thereafter, glucose rose slowly to >600 mg/dl at 15 weeks (Fig. 1), as insulin declined gradually to 0.1 ng/ml. The animals became increasingly catabolic and cachectic, dying within 18 weeks after the transplantation procedure. The chronology of the decline in plasma insulin was similar to that of obese pre-diabetic ZDF (*fa/fa*) rats (4). Examination of hematoxylin and eosin-stained liver sections revealed only minimal inflammatory changes, seemingly excluding immunological rejection.

Relationship of islet transplants to hepatic lipid content. If lipotoxic injury contributed to β -cell failure, there should be evidence of an increase in hepatic lipid content

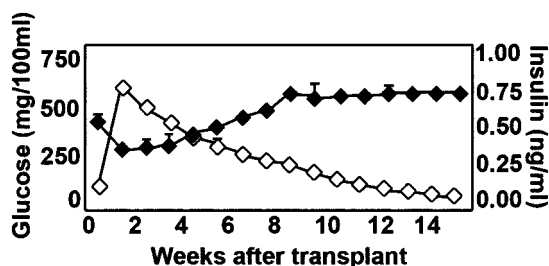


FIG. 1. Plasma insulin (\diamond) and glucose (\blacklozenge) levels in six rats with severe streptozotocin-induced diabetes in which a suboptimal dose of 1,500 islets harvested from syngeneic donors was injected via the portal vein.

surrounding the islets before β -cell failure. We therefore repeated the foregoing experiment in 18 rats but killed them 4 weeks after transplantation. Oil red O staining of liver for lipids revealed areas of intense positivity in hepatocytes immediately surrounding transplanted islets (Fig. 2A and B). The proximity of oil red O staining to islet grafts is consistent with a relationship between localized steatosis and loss of β -cells.

If loss of β -cell function was the result of lipid excess surrounding the islets, then any factor that reduces steatosis surrounding the islets should attenuate β -cell failure. Therefore, in one group of six recipients, we administered adenovirus containing leptin cDNA; the intense hyperlep-

tinemia thereby generated rapidly depletes body lipids (25). In this group, in which food intake declined by 50%, the mean plasma leptin level rose to 140 ng/ml on the seventh day and declined slowly thereafter, but remained above normal throughout the 28 days of observation (data not shown). Hepatic triacylglycerol content (Fig. 2C) averaged 2.8 ± 0.2 mg/g liver wt in ad lib-fed islet recipients, significantly above the normal level of 1.7 ± 0.02 mg/g ($P < 0.001$). It was reduced to 0.8 ± 0.03 mg/g by the hyperleptinemia. The hyperleptinemic rats were normoglycemic throughout the 4 weeks (Fig. 2D) and appeared clinically normal. In another group of six islet recipients, less profound lipopenia was induced by a 50% reduction in food intake, which matched the reduction in food intake of the hyperleptinemic group. In this diet-restricted group, hepatic triacylglycerol content averaged 1.4 ± 0.2 mg/g liver wet wt, one-half the triacylglycerol content of the ad lib-fed recipients (Fig. 2C). We assume that this lipopenic effect was caused by the 50% reduction in lipogenic substrate derived from the restricted diet.

Although reduction in hyperglycemia was modest in the diet-restricted group of recipients, they seemed much healthier than the ad lib-fed group and gained 72 ± 5 g body wt over the 4 weeks. By contrast, ad lib-fed recipients regained only 17 ± 3 g of the 38 ± 3.3 g lost after streptozotocin administration ($P < 0.001$). We attribute this difference to the slower rate of β -cell loss in the diet-restricted group.

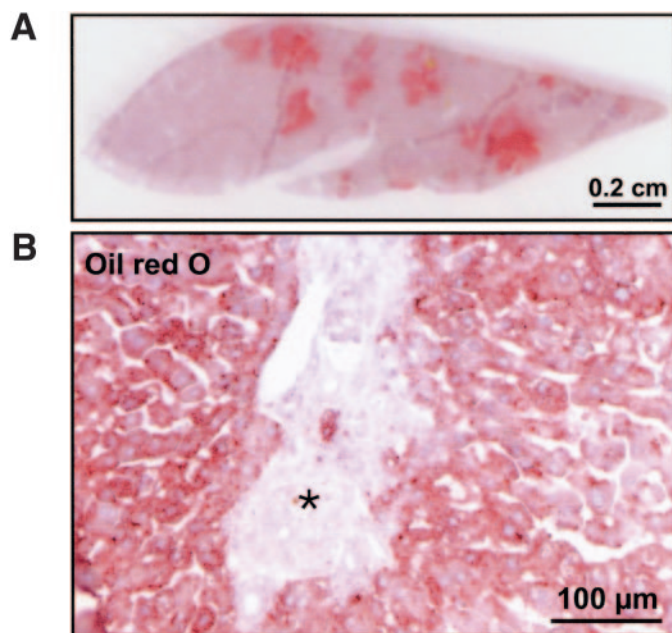
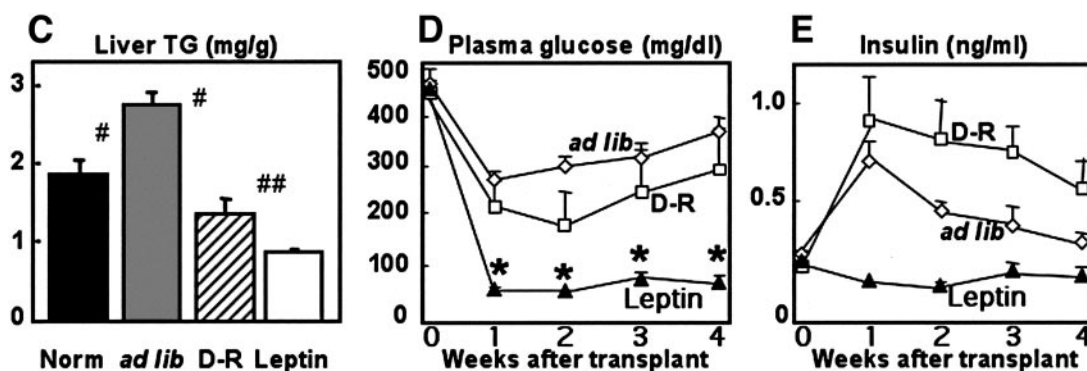


FIG. 2. A: Lipid content in islet transplants shown by oil red O staining of a liver cryosection from an ad lib-fed rat 4 weeks after islet transplantation (magnification $\times 5$). The oil red O-positive spots correspond to peri-portal regions surrounding the transplanted islets. B: Higher magnification of a portion of the section shown in A. Most of the peri-portal hepatocytes surrounding the transplanted islet (*) are heavily stained with oil red O. C: Triglyceride levels in whole liver extracts of normal (\blacksquare), ad lib-fed (\blacksquare), diet-restricted (\boxtimes), and hyperleptinemic (\square) recipients. $\#P = 0.001$; $\#\#P = 0.05$. Norm, normal nondiabetic controls; D-R, diet-restricted. D: Blood glucose levels in the three groups. $*P = 0.05$. E: Plasma insulin levels in the three groups.



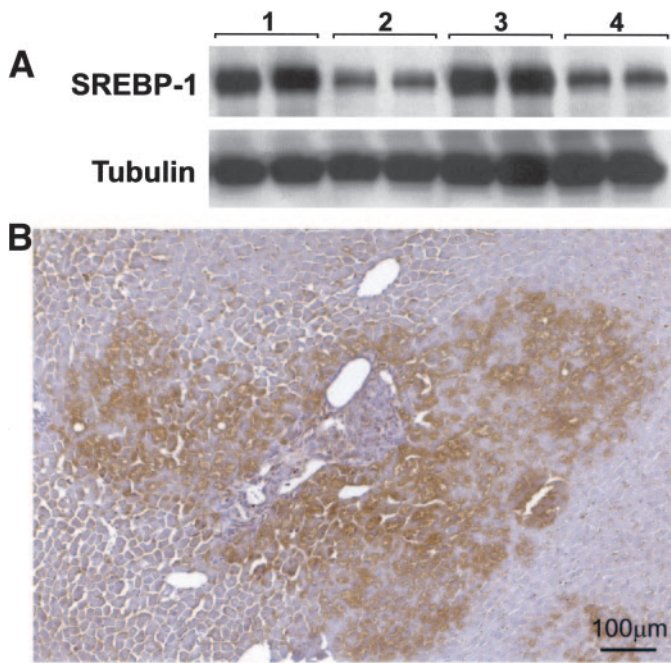


FIG. 3. A: Immunoblot analysis of SREBP-1 precursor protein in liver lysates. *Lane 1*, immunoblots of two livers from normal nondiabetic rats (blood glucose <120 mg/dl). *Lane 2*, immunoblots of two livers of suboptimally transplanted, ad lib-fed diabetic recipients of ~1,500 islets (blood glucose >400 mg/dl). *Lane 3*, immunoblots of two livers from suboptimally transplanted, diet-restricted diabetic recipients of ~1,500 islets (blood glucose <300 mg/dl). *Lane 4*, immunoblots of two livers from hyperleptinemic, normoglycemic recipients of ~1,500 islets (blood glucose <120 mg/dl). Immunoblots of tubulin were used as loading controls. Each group consisted of three rats. Immunoblots were carried out in duplicate. **B:** Immunostaining for SREBP-1 protein in the liver of a diabetic rat with failing transplants. SREBP-1-positive hepatocytes are confined to the proximity of a relatively normal-appearing transplant, the same zone in which oil red O-positive hepatocytes were identified. Each experiment was repeated three times.

Insulin levels during the 4 weeks after transplantation were highest in the diet-restricted group. In the normoglycemic leptinized group they remained at the low pretransplantation levels throughout the study period (Fig. 2E).

Molecular mechanism of lipid overaccumulation in hepatocytes surrounding the islets. The lipogenic action of insulin is mediated by the transcription factor, SREBP-1c, which upregulates the expression of lipogenic enzymes, including acetyl CoA carboxylase (ACC), SCD-1, glycerol phosphate acyl transferase, and FAS (26,27). We previously reported a 2.4-fold increase of SREBP-1c mRNA in lipid-laden livers of leptin-unresponsive ZDF rats, together with upregulation of its lipogenic target enzymes, ACC and FAS (28), suggesting that this transcription factor might be implicated in the pathogenesis of lipid overaccumulation.

To determine whether SREBP-1c is similarly involved in hepatic steatosis after transplantation and subsequent β -cell failure of normal islet transplants, we compared SREBP-1 precursor protein in liver lysates of the three groups of diabetic transplant recipients and in normal nondiabetic rats (Fig. 3A, *lane 1*). In ad lib-fed islet transplant recipients undergoing progressive failure of their grafts, hepatic SREBP-1 precursor protein was well below normal (Fig. 3A, *lane 2*), as expected. Nevertheless, in a rat with a blood glucose level of 389 mg/dl and reduced total hepatic SREBP-1 protein on immunoblotting, SREBP-1-positive hepatocytes could still be identified surrounding a relatively well-preserved islet, whereas

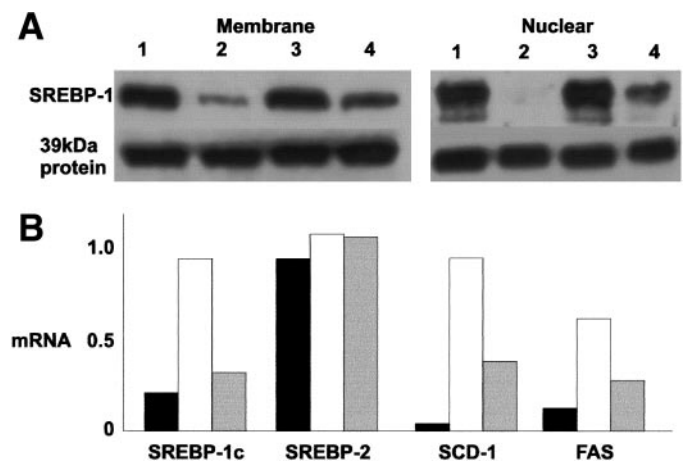


FIG. 4. A: Immunoblot analysis of precursor (membrane) and mature (nuclear) SREBP-1 protein in a pool of three livers obtained from each group. *Lane 1*, immunoblot of livers of normal nondiabetic rats (blood glucose <120 mg/dl). *Lane 2*, immunoblot of livers of untransplanted rats with uncontrolled streptozotocin-induced diabetes (blood glucose >500 mg/dl). *Lane 3*, immunoblot of livers from normoglycemic streptozotocin-induced diabetic rats after transplantation of ~2,000 islets (blood glucose <100 mg/dl). *Lane 4*, immunoblot of livers from hyperleptinemic, normoglycemic streptozotocin-induced diabetic rats after transplantation of ~2,000 islets (blood glucose <120 mg/dl). Immunoblots of 39-kDa protein (RAP) were used as loading controls. **B:** Comparison by real-time RT-PCR of mRNA of SREBP-1c and two target enzymes, SCD-1 and FAS. Equal amounts of RNA from liver of three rats were pooled for real-time RT-PCR analysis. Cyclophilin was used as the invariant control. Values represent the amount of mRNA relative to that of the normal, nondiabetic control rats, arbitrarily assigned a value of 1. Severely diabetic untransplanted rats (■), normoglycemic recipients of ~2,000 islets (□), and hyperleptinemic, normoglycemic recipients (▣).

more remotely situated hepatocytes were SREBP-1 negative (Fig. 3B). The SREBP-1-positive cells were virtually superimposable upon oil red O-positive hepatocytes of an adjacent section. In three other livers from the same group, SREBP-1 staining surrounding islets was extremely weak and was therefore considered to be negative.

In islet recipient rats in which severe diabetes had been attenuated by 50% diet restriction, total SREBP-1 was almost normal on immunoblotting (Fig. 3A, *lane 3*). This suggests that the reduction in triacylglycerol content in their livers was the result of dietary restriction of lipogenic substrate rather than diminished SREBP-1 expression. In hyperleptinemic recipients with normoglycemia (blood glucose <120 mg/dl), SREBP-1 was very low (Fig. 3A, *lane 4*). Thus, when insulin is low because of insulin deficiency or because of its suppression by hyperleptinemia, SREBP-1 protein is reduced and lipid synthesis is minimal.

Because SREBP isoforms become transcriptionally active only after processing and transport of the active product into nuclei (26,27), we compared both membrane and nuclear SREBP-1 proteins in the liver of a normal, nondiabetic rat (Fig. 4A, *lane 1*) with an untransplanted, untreated, severely catabolic streptozotocin-induced diabetic rat (blood glucose >500 mg/dl) (Fig. 4A, *lane 2*) and a severely catabolic streptozotocin-induced diabetic rat made normoglycemic by transplanting an optimal dose of ~2,000 islets (blood glucose <100 mg/dl) (Fig. 4A, *lane 3*). In the untransplanted diabetic group, nuclear SREBP-1 was virtually undetectable, and the membrane fraction was markedly reduced; in the diabetic rats rendered normoglycemic by an optimal dose of islets, both membrane and nuclear fractions of SREBP-1 protein were as high as in nondiabetic control rats, in association with a triacylglycerol content of 3.1 ± 0.2 mg/g liver, which is

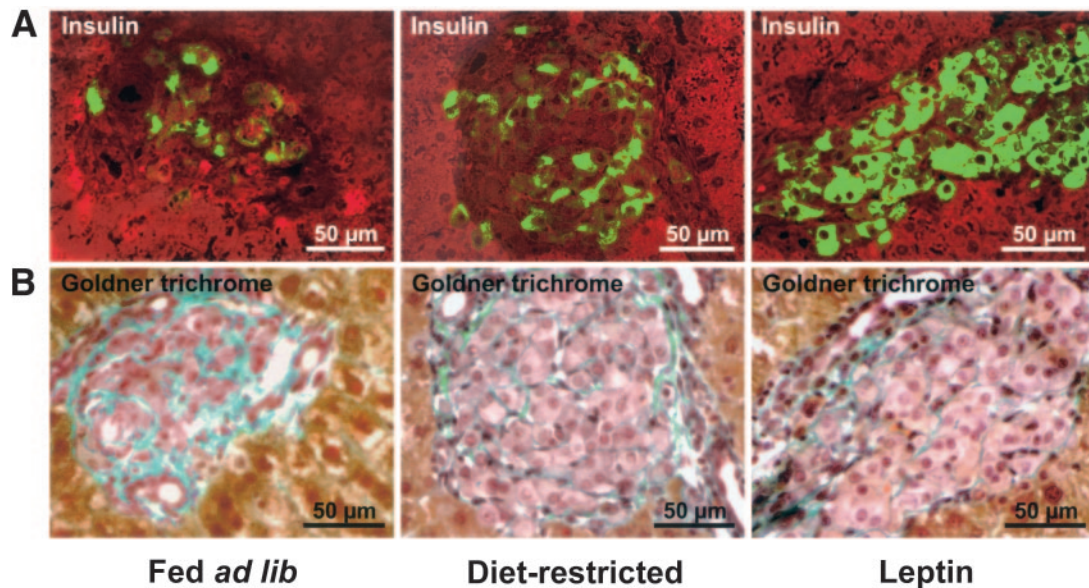


FIG. 5. Pairs of consecutive paraffin sections of Bouin-fixed liver showing islets transplanted in portal regions. **A:** Immunofluorescence staining with insulin antibodies showing a representative islet from ad lib-fed, diet-restricted, and leptin-treated animals. The islet from the first two groups shows a reduced number of insulin cells. Quantitative analysis of insulin-positive cells appears in RESULTS. **B:** Goldner trichrome staining in ad lib-fed animals reveals marked distortion of islets by thick bands of collagen. This was reduced in the diet-restricted animals and was minimal in the leptinized rats. Quantitative analysis of fibrosis appears in RESULTS.

higher than in the ad lib-fed, suboptimally transplanted rats of Fig. 2C. In optimally transplanted normoglycemic recipients made hyperleptinemic, both membrane and nuclear SREBP-1 protein were profoundly reduced (Fig. 4A, lane 4), consistent with earlier evidence that hyperleptinemia downregulates SREBP-1c mRNA in liver (28). Interestingly, these optimally transplanted normoglycemic recipients remained normoglycemic for >21 weeks after transplantation, whereas the residual hyperglycemia of suboptimally transplanted recipients worsened in 4 weeks. This strongly suggests that accelerated β -cell failure in the latter rats resulted from greater abundance of lipogenic substrate provided by elevated glucose levels or from direct glucotoxic effect or from both, as proposed by Robertson et al. (23) and Harmon et al. (23).

The mRNA of SREBP-1c, expressed as fold difference from nondiabetic controls, followed the same pattern. In untransplanted streptozotocin-induced diabetic rats, liver SREBP-1c mRNA was 20% of the level in normal nondiabetic liver (Fig. 4B). In recipients with normoglycemia after optimal dose islet transplantation, it had risen to normal, but in normoglycemic, hyperleptinemic recipients, it was suppressed to 35% of normal. By contrast, the mRNA of SREBP-2, a transcription factor involved mostly in cholesterologenesis, was the same in all groups (Fig. 4B).

The mRNA of two of the SREBP-1c target enzymes, SCD-1 and FAS, reflected the foregoing changes in the expression of their transcription factor. Both were low in livers of insulin-deficient, uncontrolled diabetic rats, and both rose to control or near-control levels after high-dose islet transplantation. Hyperleptinemia reduced these increments (Fig. 4B). Thus, all expression patterns of SREBP-1c mRNA and SREBP-1 protein were consistent with a role in insulin-mediated lipogenesis and leptin-induced antilipogenesis.

Relationship of triacylglycerol content to β -cell loss, islet fibrosis, and diabetes. To obtain further evidence that reduction in lipid accumulation will protect against β -cell loss, we compared the area of insulin-positive cells

per $1 \times 10^6 \mu\text{m}^2$ area in three randomly selected liver sections in three animals from each group of islet transplant recipients. In ad lib-fed recipients with failing transplants and uncontrolled diabetes, only $114 \pm 12 \mu\text{m}^2$ insulin-positive cells were present per $1 \times 10^6 \mu\text{m}^2$ liver. In diet-restricted recipients, with significantly reduced triacylglycerol content, the insulin-positive area was 3.7-fold greater, averaging $423 \pm 128 \mu\text{m}^2$ per $1 \times 10^6 \mu\text{m}^2$ liver. In the leptinized group with the lowest triacylglycerol content, the insulin-positive area was 24-fold greater than in the ad lib-fed rats, averaging $2,806 \pm 377 \mu\text{m}^2$ per $1 \times 10^6 \mu\text{m}^2$ liver ($P < 0.001$ vs. the other groups). Figure 5A displays a representative islet from each group.

In the ad lib-fed group, islets were markedly distorted, and β -cells were replaced by thick bands of collagen with a volume density of 0.29 ± 0.03 ($n = 10$ islets). Fibrosis was less in the diet-restricted group, measuring only 0.11 ± 0.01 ($n = 8$ islets). In the hyperleptinemic recipients, it was virtually absent, with a volume density of 0.05 ± 0.01 ($n = 8$ islets). Thus, β -cell depletion and fibrosis were most severe in the islets with the greatest lipid exposure, and both abnormalities were reduced by diet restriction and hyperleptinemia, consistent with a lipid-related mechanism. Figure 5B displays a representative islet from each group. Note that the fibrosis resembles that reported in islets of type 2 diabetic ZDF rats (6), in which diet restriction also prevented β -cell loss, fibrosis, and diabetes (7). Poststeatotic fibrosis is a familiar occurrence in nonalcoholic steatohepatitis and can occur in other organs, including the heart (29).

Apoptosis as the cause of the β -cell loss. Previously, we invoked lipid-induced apoptosis as the cause of β -cell destruction and type 2 diabetes in obese ZDF rats (lipopapoptosis) (30). This was based on the association of high lipid content with increased DNA fragmentation and severe mitochondrial alterations of β -cells (6). To determine whether lipid-related destruction of β -cells noted in islet transplants is also involved an apoptotic mechanism, we used TUNEL staining of the livers as an index of apoptosis. In ad lib-fed rats, in which β -cells were very sparse,

TUNEL-positive cells were virtually absent, presumably because affected β -cells had already disappeared by the time of killing. However, in three liver sections from three diet-restricted rats, in which insulin-positive β -cells were 3.7-fold more abundant than in the ad lib-fed group, TUNEL-positive cells averaged 2 ± 0.2 cells per portal area. In hyperleptinemic recipient rats, in which β -cells were 24 times as abundant as in the ad lib-fed group, apoptotic granules averaged only 0.8 ± 0.1 per portal area ($P < 0.01$) (data not shown).

DISCUSSION

Despite the early promise of islet transplantation as a cure for type 1 diabetes, the results have been less than anticipated. In an international, multicenter trial using the so-called Edmonton protocol, only 13% of transplant recipients were insulin independent at 2 years after the procedure (3). The cause of this high rate of late transplant failure has not been elucidated, but immunorejection, toxicity of immunosuppression agents, and aberrant blood supply to the intrahepatic islet grafts have been considered. The possibility of lipotoxicity (4), a metabolic form of β -cell rejection, had not been entertained previously.

“Lipotoxicity” of β -cells has been proposed as a cause of non-insulin-dependent type 2 diabetes based on studies of obese ZDF rats (30), in which hyperphagia and hyperinsulinemia are followed by overaccumulation of lipids in the islets and β -cell hyperplasia (5) and then by β -cell apoptosis (24) and overt type 2 diabetes (4). As in the present model, β -cell loss and diabetes in ZDF rats could be prevented by reduction of ectopic lipid accumulation by caloric restriction (7), thiazolidinediones (6), and 5-aminoimidazole-4-carboxamide-1- β -D-ribofuranoside (31). In addition, phloridzin treatment can prevent ZDF diabetes (21), presumably by depleting lipogenic substrate via a renal glucose leak, thereby lowering islet triacylglycerol, and by suppressing insulin, the lipogenic hormone, while stimulating antilipogenic glucagon (32). In the ZDF model, lipotoxicity has been attributed to the lack of leptin action resulting from a loss-of-function mutation in the leptin receptor; this results in a severe lipid accumulation that ultimately disables and kills β -cells through apoptosis. It seemed plausible that the slow, late β -cell destruction in intraportal islet grafts might not involve the same metabolic pathology. We reasoned that in recipients of intraportal islet transplants, the combination of systemic hyperglycemia and high portal vein levels of nutrients and incretins would increase the secretion of insulin by the grafts, creating a surrounding zone of overinsulinized hepatocytes with upregulation of SREBP-1c and its target enzymes of lipogenesis. Lipoprotein lipase of the islets (18) would hydrolyze the increased levels of triacylglycerol released by surrounding hepatocytes, thereby exposing their β -cells to increasing fatty acids. This, we imagined, would cause lipotoxicity of β -cells and failure of the islet grafts, particularly if there was accompanying hyperglycemia (33). If so, interventions that reduce lipogenesis should prevent the damage.

These predictions were borne out. In livers of normoglycemic rats, in which severe diabetes had been reversed by optimal dose islet transplantation, the mRNA of the lipogenic transcription factor SREBP-1c was far above that of severely diabetic, untransplanted controls, as was the SREBP-1 protein in nuclear fractions of their liver. mRNA of its target enzymes, SCD-1 and FAS, was also increased from the depressed levels of untransplanted

CHRONIC OVERNUTRITION

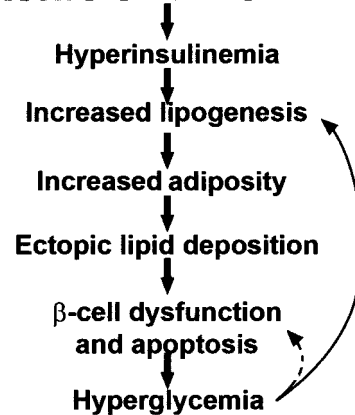


FIG. 6. Putative pathogenesis of lipotoxic type 2 diabetes based on this study. We postulate that type 2 diabetes can be caused by overstimulation of insulin secretion by chronic overnutrition. The combined caloric excess and hyperinsulinemia induce excessive SREBP-1c-mediated lipogenesis in liver and adipocytes that provide a source of ectopic lipids to islets and other organs. Fatty acids derivatives from imported fatty acids or from locally synthesized fatty acids can impair and destroy β -cells, thereby causing overt type 2 diabetes. The coexistence of hyperglycemia, as in the suboptimally transplanted streptozotocin-induced diabetic islet recipients of this study, can accelerate the lipotoxicity by providing glucose, a substrate for lipogenesis, and/or by causing direct damage (broken arrow).

diabetic rats. Despite the marked reduction in SREBP-1 protein in extracts of livers from ad lib-fed rats after failure of their transplanted islets, hepatocytes immediately surrounding some islets still remained oil red O- and SREBP-1-positive, suggesting residual localized insulin action. Most islets within this steatotic zone were clearly abnormal, with a sharp reduction in insulin-positive β -cells and replacement by thick collagen strands reminiscent of the poststeatotic fibrosis observed in islets of ZDF rats (6), in heart (34), and in liver. The mildness of the inflammatory reaction seemed to exclude immunological rejection as the cause of the β -cell loss.

In contrast to these normoglycemic, optimally transplanted recipients in which blood glucose levels remained normal until 21 to 28 weeks after the procedure, combined glucose and lipid overload in suboptimally transplanted recipients appears to be far more toxic than lipid overload alone, in support of the gluco-lipotoxicity concept (23,33) (Fig. 6). In suboptimally transplanted recipients with moderate hyperglycemia, the metabolic deterioration occurred within 4 weeks. We speculate that the uncorrected hyperglycemia provided a greater abundance of lipogenic substrate, while stimulating insulin, the lipogenic hormone, and suppressing glucagon, an antilipogenic hormone. Alternatively, hyperglycemia itself might have caused direct injury to β -cells, although the marked fibrosis strongly argues for lipotoxicity.

The possible role of lipotoxic destruction in human islet transplants is supported by magnetic resonance images of such patients showing accumulation of intrahepatic fat surrounding the graft sites (12) not unlike those found by oil red O staining in this study (Fig. 2B). This raises the possibility that lipopenic interventions might extend survival of intraportal islet transplants in humans, as they did in rats.

In summary, this study demonstrates, first, that transplantation of islets into the liver is accompanied by insulin-stimulated SREBP-1c-mediated lipogenesis followed by destruction of β -cells. Second, it shows that the destruction can be prevented by reducing the availability

of lipogenic substrates such as glucose and lipids. It implies that a key pathogenic abnormality in the failure of intrahepatic islet grafts may well be insulin-mediated lipotoxicity due to excessive insulin-stimulated lipogenesis. Its elimination by hyperleptinemia prevents the failure. This evidence that lipid excess can destroy normal β -cells is compatible with the concept of lipotoxicity as a factor in type 2 diabetes (30), as depicted in Fig. 6.

ACKNOWLEDGMENTS

R.H.U. received grants from the National Institute of Diabetes and Digestive and Kidney Diseases, the Department of Veterans Affairs Merit Review, the Juvenile Diabetes Research Foundation, and Takeda Pharmaceuticals. L.O. received a grant from the Swiss National Science Foundation. Y.K.B. received support from National Institutes of Health Grant HL-20948 to the Brown-Goldstein laboratory.

We are grateful to Sara Kay McCorkle, Ariane Widmer, and Marie Ebrahim Malek for technical assistance; Nadine Dupont for image processing; and Peggy McCravy for secretarial work. We thank James A. Richardson and John Shelton for their work in the UT Southwestern Molecular Pathology Core. Chris Newgard, Daniel Foster, Scott Grundy, and Lorenzo Orci provided critical reviews.

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