Subcutaneous, Isogeneic Transplantation of Duct-ligated Pancreas in Streptozotocin-Diabetic Mice

Relationships Between Carbohydrate Tolerance and Hormone Content in Transplant or Host Pancreas

ROBERT C. KRAMP AND IAN M. BURR

SUMMARY

Mice with subcutaneous, isogeneic transplants of duct-ligated pancreas from either two or five donors displayed impaired glucose tolerance to gastric or intravenous glucose, or to an overnight fast followed by a 15-min meal of mouse food. Compared with peripheral insulin responses in normal controls, those of transplant recipients were less after gastric or intravenous glucose, but no different after the meal. Despite normalization of peak insulin levels in the peripheral circulation of isografted mice, glucose clearance was still impaired and this probably resulted, in part, from a relative insufficiency of insulin in the portal circulation. Results of glucose tolerance tests, after transplant recipients consumed a relatively small amount of food, suggested that near-normal glucose homeostasis may be present for these mice under normal feeding conditions.

In mice that received incremental doses of streptozotocin, impaired glucose tolerance to a meal was observed if pancreatic insulin content fell below 26% of normal. We failed to show a similar relationship between glucose tolerance and pancreatic insulin content in transplant recipients since all showed impaired glucose tolerance. Despite the initial transplantation of different amounts of islet tissue, at termination insulin content (transplant plus endogenous pancreas) was essentially the same for all recipients, totaling 19–22% of that found in a normal mouse pancreas.

DIABETES *30*:857–864, October 1981.

Pancreatic transplantation for management of diabetes mellitus requires a knowledge of the pancreatic content of hormones or islet mass necessary for optimal control of plasma glucose. This report extends previous studies[1,2] using subcutaneous, isogeneic transplants of duct-ligated pancreas in streptozotocin-treated mice to further define the relationship between pancreatic hormone content and glucose tolerance. Mice were transplanted with duct-ligated pancreata from either two or five donors, and their tolerance to intravenous or gastric glucose administration or to different amounts of mouse food was examined. The relationship between hormone content and glucose tolerance was also studied in mice that received incremental doses of streptozotocin.

MATERIALS AND METHODS

Female, 5-mo-old mice (C57Bl/Cum ♀ × C3H/Anf Cum ♂, F_1, called $BC3F_1$) purchased from Cumberland View Farms (Clinton, Tennessee) were used as donors or recipients. This hybrid was chosen because it provides the highest percentage of useable donor tissue preparations after pancreatic duct ligation,[1,3] and it shows the most rapid recovery from hyperglycemia after transplantation[4] compared with other strains or hybrids we have used.

Transplantation of duct-ligated pancreas and injection of streptozotocin. Ligation of pancreatic ducts in donors and subcutaneous, isogeneic transplantation of duct-ligated pancreas were performed as previously described.[1] A silk thread was tied around the donor pancreas midway between duodenum and spleen, dividing the gland into roughly equal parts by weight. About 8 wk later, the pancreatic segment between ligature and spleen was removed, cut into small fragments, and placed into subcutaneous pockets created in the axillary regions of the recipient. Recipients were made diabetic by i.v. injection of streptozotocin (STZ) (lot no. 60,273-1, U-9889, a gift of W. E. Dulin, the Upjohn Company, Kalamazoo, Michigan) dissolved in phosphate-buffered saline, acidified to pH 4.5 with 0.05 M citric acid. An initial nondiabetogenic dose of streptozotocin, 120 mg/kg mouse, was followed 2 wk later by a dose of 240 mg/kg mouse. Although in earlier studies[1,4] a single diabetogenic dose was used, recently[2] we observed that the two-dose regimen resulted in a greater incidence of hyperglycemia. Two weeks after the last injection of streptozoto-

From the Vanderbilt University School of Medicine, Department of Pediatrics, Nashville, Tennessee, and Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Address reprint requests to Robert C. Kramp, Ph.D., Virginia Polytechnic Institute and State University, Department of Biology, Blacksburg, Virginia 24061.

Received for publication 18 February 1981.

cin, 20 diabetic recipients each received duct-ligated pancreata from 2 donors, and 15 recipients each received duct-ligated pancreata from 5 donors. Fifteen sham-operated mice were treated in the same surgical manner as transplant recipients but received no pancreatic tissue. Seventeen aged-matched mice served as normal, nondiabetic controls.

Additional mice were given a single intravenous injection of streptozotocin at doses of 140, 160, 180, 200, 220, 240, or 260 mg/kg mouse. Each dose was applied to groups of three or four mice.

Experimental mice were separated according to treatment and housed 3–5 per cage. At intervals before and up to 23 wk after transplantation, body weights were taken and blood samples were obtained by retroorbital puncture from 7-h fasted, unanesthetized mice. The plasma was assayed for glucose and immunoreactive insulin (IRI). During the first 9 wk after transplantation, water consumption was estimated thrice weekly: water bottles were weighed after 48 h, and the net difference in weight was divided by the number of mice per cage.

Carbohydrate tolerance tests. Transplant recipients and groups of nondiabetic controls were tested for carbohydrate tolerance over a 2-wk period at three intervals (5 and 6, 11 and 12, and 17 and 18 wk) after transplantation. During the first week of each interval, half the recipients, including mice transplanted with two duct-ligated pancreata and those transplanted with five, were given an intravenous (i.v.) glucose injection (1.0 g/kg mouse) via the lateral tail vein. The remaining half were given a gastric glucose load (3.0 g/kg mouse) administered with blunt-ended, oral administration needles (Popper and Sons, Inc., New Hyde Park, New York). The i.v. and gastric glucose tolerance tests were alternated between grouped recipients at the various intervals after transplantation. Mice were fasted from 0700 to 1400 h before testing. During the second week of each interval, all mice were tested for meal tolerance. Mice were caged four or five per cage, fasted overnight for 18 h, and then exposed to Wayne Lab Blox mouse food, which contains 24.5% protein, 4.1% fat, 50.2% nitrogen-free extract, and 4.1 kcal/g-gross energy. Food was weighed before and after the meal, and divided by the number of mice per cage to estimate the amount of food consumed per mouse. Though it was not possible to guarantee uniform comsumption of food, we assumed that vagaries in food comsumption among individual mice would be the same between normal control and treated groups. In pilot studies, we observed that fasted mice began eating as soon as food was available, and that the average amount of food consumed was the same whether mice were caged individually or in small groups. We also found that normal mice, with few exceptions, showed similar insulin responses after a specific period (15 min or less) of exposure to food.

In preliminary trials, we determined that blood samples obtained at two crucial times during each of the tolerance tests, at 3 and 30 min after i.v. glucose and at 15 and 60 min after either gastric glucose or food ingestion, provided adequate data for comparing test performances between normal mice and transplant recipients. To further reduce the frequency of blood sampling, samples for basal plasma glucose and IRI were obtained 3 or 4 days before tolerance tests in mice fasted for 7 h.

Hormone extraction and radioimmunoassays. As previously described,[4] transplants of duct-ligated pancreas could be grossly distinguished in the subcutaneous transplant site as a yellowish, fibrotic and fatty mass which appeared well vascularized. This tissue containing the presumed transplanted islets and the recipient's own pancreas were excised. Then each tissue was extracted for hormones twice by sonification in a total of 6 ml of 0.15 N HCl in 75% ethanol. Aliquots of the extracts were diluted to final dilutions ranging from 1:600 to 1:12000, with 0.2 M glycine buffer containing 0.25% bovine serum albumin that was especially prepared for radioimmunoassays (Sigma Chemical Corp., St. Louis, Missouri). Immunoreactive insulin (IRI) and glucagon (IRG) were measured by the single-antibody, charcoal separation method of Herbert et al.[5] using guinea pig anti-porcine glucagon serum (gift of Dr. R. H. Unger, Southwestern Medical School, University of Texas, Dallas, Texas) and rat insulin or porcine glucagon as standards. The sensitivity of the radioimmunoassay for IRI (0.125 ng/ml) and IRG (0.030 ng/ml) combined with the lowest dilution of acid alcohol extracts (1:600) yielded a minimum detectability of 75 and 18 ng for IRI and IRG, respectively. A micromodification[1] of the radioimmunoassay was used for measuring IRI in duplicate samples of 10 μl of plasma.

RESULTS

Recovery from hyperglycemia in streptozotocin-treated mice after receiving subcutaneous isogeneic transplants of either two or five duct-ligated pancreata (Figure 1). Recovery from hyperglycemia was most rapid in those mice receiving five duct-ligated pancreata; normoglycemia was attained within 3–5 wk. Though mice transplanted with two duct-ligated pancreata showed plasma glucose below that of sham-operated mice at 2 wk, complete recovery from hyperglycemia in the majority of these recipients did not occur until 9 wk. Two mice in this group failed to recover from hyperglycemia during the experiment. Two others that had recovered died of unknown causes at 5 and 20 wk after transplantation. At 13 wk, plasma glucose was not significantly different between mice transplanted with two or five duct-ligated pancreata, and the plasma glucose of both groups was within the normoglycemic range. Once recovered from hyperglycemia, transplant recipients did not revert to the hyperglycemic state. Thrice weekly determinations of water consumption confirmed the differences in the rate of recovery from experimental diabetes between the two groups of isografted mice. Polydipsia in mice receiving five duct-ligated pancreata abated by 5 wk; that in mice receiving two duct-ligated pancreata by 9 wk. Sham-operated mice remained severely hyperglycemic and polydipsic. Between 10 and 12 wk, 13 of 15 sham-operated mice died.

Carbohydrate tolerance in streptozotocin-treated mice at intervals after receiving subcutaneous, isogeneic transplants of either two or five duct-ligated pancreata (Figure 2 and Table 1). Though all transplant recipients were tested for carbohydrate tolerance at each interval after transplantation, we present here data only for those mice that showed basal plasma glucose below 225 mg/dl 3 or 4 days before tolerance testing. These data were selected because we were interested primarily in comparing glucose

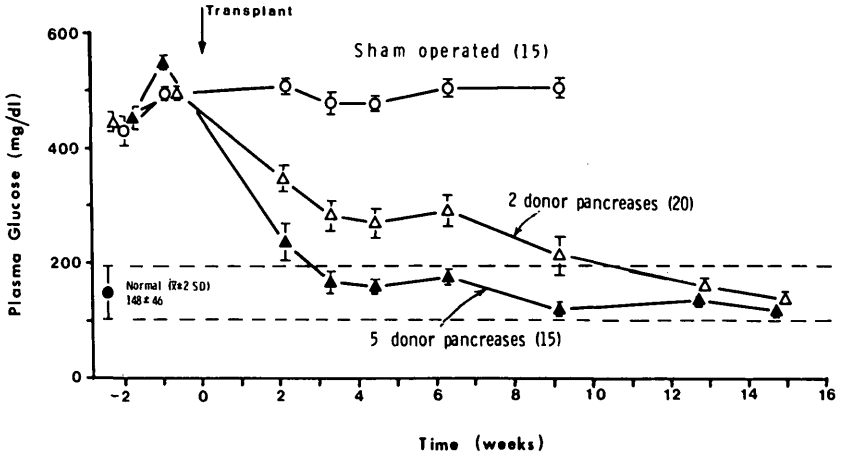

FIGURE 1. Plasma glucose ($\bar{X} \pm$ SEM) of streptozotocin-treated mice after sham operation (0) or after receiving subcutaneous, isogeneic transplants of either two (△) or five (▲) duct-ligated pancreata. Dashed lines represent $\bar{X} \pm 2$ SD of plasma glucose of normal mice.

tolerance between normal mice and transplant recipients that had recovered from hyperglycemia.

Figure 2 shows the results of tolerance tests performed on nondiabetic controls and transplant recipients 11 and 12 wk after transplantation, when most of the recipients had recovered. After intravenous or gastric glucose, IRI responses of isografted mice were significantly below those of nondiabetics, and their glucose tolerance was impaired. In contrast to exogenous glucose administration, IRI responses after a 15-min meal were not significantly different between isografted and normal mice. Even though there was no difference in peak IRI levels, plasma glucose of transplant recipients was substantially higher than that of nondiabetics at 15 min, and was still elevated at 60 min.

The results of these tests performed at 11 and 12 wk were typical for tolerance tests performed at 5 and 6 wk or at 17 and 18 wk after transplantation. Table 1 presents results of all tolerance tests only for those times during each test that were most important for comparing test performances among the two isografted groups and nondiabetic controls. In each type of tolerance test, the differences in plasma glucose and IRI responses among transplant recipients and nondiabetics did not change appreciably among the different intervals after transplantation. In comparison to nondiabetic controls, transplant recipients showed significantly lower plasma IRI responses after i.v. or gastric glucose loads, the same plasma IRI responses after a 15-min meal, and impaired glucose tolerance after all three types of carbohydrate challenges. In the meal tolerance test performed at 6 wk, however, IRI responses were lower in isografted mice than in nondiabetics. In general, there were no significant differences in glucose tolerance and IRI responses between recipients with either two or five duct-ligated pancreata. One notable exception was in the gastric glucose tolerance test at 5 wk in which mice transplanted with five duct-ligated pancreata displayed significantly higher IRI responses at 15 min even though there was no difference in glucose levels between the two isografted groups.

In normal mice, peak IRI responses after gastric or i.v. glucose or after a 15-min meal averaged between 1 and 3 ng/ml with no substantial differences observed among the different tests, except after the gastric glucose tolerance test at 6 wk in which IRI responses averaged over 4 ng/ml. By contrast, recipients whose subcutaneous transplants re-

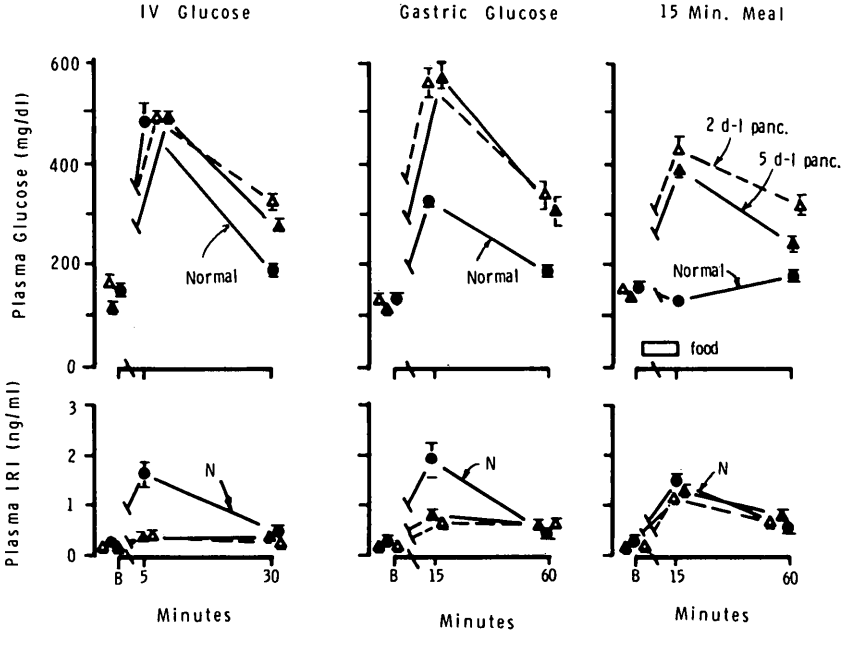

FIGURE 2. Plasma glucose ($\bar{X} \pm$ SEM) or immunoreactive insulin (IRI) after intravenous or gastric administration of glucose, or after a 15-min exposure to food (15-min meal) in normal mice (●) and in streptozotocin-treated mice 10–12 wk after receiving subcutaneous, isogeneic transplants of either two (△) or five (▲) duct-ligated pancreata. The "B" on the time scale represents basal plasma glucose and IRI in 7-h fasted mice obtained 3 or 4 days before tolerance tests. Sample sizes are given in Table 1.

TABLE 1
Plasma glucose (G, mg/dl) and immunoreactive insulin (IRI, ng/ml) at various times after gastric or intravenous (i.v.) administration of glucose* or after a 15-min meal at intervals after streptozotocin-treated mice were transplanted subcutaneously with either two or five duct-ligated (d-l) pancreata†

Weeks after transplant	Treatment group	Sample size‡	i.v. Glucose		Gastric glucose		15-min meal	
			G 30 min	IRI 3 min	G 15 min	IRI 15 min	G 15 min	IRI 15 min
5–6	Nondiabetics	6, 6, 12	192 ± 14	1.38 ± 0.24	296 ± 23	4.58 ± 0.81	124 ± 5	1.71 ± 0.22
	2 d-l pancreas	5, 4, 8	294 ± 19	0.40 ± 0.13	584 ± 24	0.58 ± 0.03	345 ± 16	0.83 ± 0.42
	5 d-l pancreas	6, 9, 15	276 ± 13	0.55 ± 0.07	580 ± 19	1.22 ± 0.17	384 ± 18	1.06 ± 0.12
	P§ 1 vs. 3		<0.001	<0.005	<0.001	<0.001	<0.001	<0.010
	P 2 vs. 3		NS	NS	NS	<0.001	NS	NS
11–12	Nondiabetics	6, 5, 17	190 ± 12	1.64 ± 0.26	327 ± 10	1.92 ± 0.35	127 ± 4	1.50 ± 0.15
	2 d-l pancreas	7, 8, 15	314 ± 17	0.38 ± 0.04	564 ± 31	0.63 ± 0.06	429 ± 26	1.04 ± 0.07
	5 d-l pancreas	9, 6, 15	279 ± 15	0.36 ± 0.04	575 ± 26	0.79 ± 0.08	386 ± 16	1.31 ± 0.15
	P 1 vs. 3		<0.001	<0.001	<0.001	<0.010	<0.001	NS
	P 2 vs. 3		<0.050	NS	NS	NS	NS	NS
17–18	Nondiabetics	6, 7, 17	209 ± 13	0.86 ± 0.10	375 ± 30	3.04 ± 0.50	148 ± 7	1.83 ± 0.26
	2 d-l pancreas	8, 7, 17	290 ± 14	0.39 ± 0.05	558 ± 28	0.79 ± 0.15	307 ± 14	1.54 ± 0.08
	5 d-l pancreas	6, 9, 15	343 ± 16	0.45 ± 0.10	483 ± 25	1.22 ± 0.24	307 ± 18	1.48 ± 0.12
	P 1 vs. 3		<0.001	<0.020	<0.020	<0.005	<0.001	NS
	P 2 vs. 3		<0.020	NS	NS	NS	NS	NS

* 1.0 g glucose/kg mouse, intravenously, or 3.0 g glucose/kg mouse, gastrically.
† Data ($\bar{X}$ ± SEM for all values) are included only for those isografted mice that showed basal plasma glucose (7-h fasted) below 225 mg/dl 4 days before tolerance testing.
‡ Sample sizes for the i.v., oral, and 15-min meal tolerance tests, respectively.
§ Significant differences calculated by Student's *t* test, for unpaired groups. NS = nonsignificant ($P > 0.05$).

lease IRI directly into the systemic circulation generally showed IRI responses that were significantly higher after either gastric glucose or food ingestion than after i.v. glucose.

Effect of amount of food consumed on plasma glucose and IRI (Table 2). Results obtained at 20 wk after transplantation on the responses after a 15-min meal (as shown in Table 2) were similar to the data for a 15-min meal obtained earlier in the study (Table 1). Plasma glucose was substantially higher in transplant recipients than in nondiabetic controls, whereas plasma IRI responses were similar. However, in this particular test at 20 wk, the insulin responses of mice with 5 duct-ligated pancreata were significantly below those of nondiabetics. During a 3-min exposure to food, both isografted mice and nondiabetics consumed about one-half as much food as during a 15-min meal. Plasma glucose of isografted mice, though quite variable after either meal, tended to be lower after mice consumed the smaller amount of food. Indeed, the averaged plasma glucose of mice transplanted with 2 duct-ligated pancreata was not significantly different from that of nondiabetics. Insulin responses after the 3-min meal were not different between either isografted group and nondiabetics.

Content of hormones in the pancreas and transplants of STZ-treated mice 23 wk after transplantation with either two or five duct-ligated pancreata (Table 3). In transplants, IRG content in mice receiving two duct-ligated pancreata was only 60% of that in mice receiving five duct-ligated pancreata, but IRI content was the same for both groups. As footnoted in Table 3, IRI in the two mice transplanted with two duct-ligated pancreata that failed to recover from hyperglycemia was only one-seventh of that in transplants of mice that did recover.

In the endogenous pancreas of transplant recipients, IRI was 6–9% of that in the pancreas of nondiabetic controls,

TABLE 2
Effect of the amount of mouse food consumed* on plasma glucose and immunoreactive insulin (IRI)† in streptozotocin-treated mice 20 wk after receiving subcutaneous transplants of either two or five duct-ligated (d-l) pancreas

Group	3-min meal				15-min meal			
	No. of mice	Food consumed (g)	Glucose (mg/dl)	IRI (ng/ml)	No. of mice	Food consumed (g)	Glucose (mg/dl)	IRI (ng/ml)
Nondiabetic	6	0.17	173 ± 10	0.71 ± 0.17	6	0.48	152 ± 12	2.84 ± 0.52‡
2 d-l pancreas	8	0.21	226 ± 28	0.68 ± 0.10	8	0.46	318 ± 53‖	1.74 ± 0.28‡
5 d-l pancreas	9	0.16	265 ± 15‖	0.78 ± 0.13	6	0.31	360 ± 42‖§	1.04 ± 0.21¶

* Mice were starved overnight for 18 h and then exposed to mouse food for 3 min or 15 min.
† Plasma was collected 15 min after mice were first presented with food.
‡ Significantly different from corresponding value under 3-min meal, $P < 0.005$.
§ Significantly different from corresponding value under 3-min meal, $P < 0.050$.
‖ Significantly different from nondiabetics, $P < 0.001$ or $P < 0.005$.
¶ Significantly different from nondiabetics, $P < 0.010$.

TABLE 3
Immunoreactive insulin (IRI) and glucagon (IRG) in the pancreas and subcutaneous transplants of streptozotocin-treated mice 23 wk after receiving either two or five duct-ligated (d-l) pancreata*

Group	No. of mice	Pancreas Tissue (mg)	Pancreas IRI (μg)	Pancreas IRG (ng)	Transplant Tissue (mg)	Transplant IRI (μg)	Transplant IRG (ng)
Nondiabetic	13	329 ± 10	43.23 ± 2.90	1626 ± 146		(Not transplanted)	
2 d-l pancreas†	16	359 ± 13	3.75 ± 0.16	560 ± 32	438 ± 35	5.67 ± 0.35	323 ± 32
5 d-l pancreas	15	320 ± 11	2.77 ± 0.18	456 ± 43	463 ± 24	5.62 ± 0.25	516 ± 54
Sham-operated	2	344	0.69	2280		(Not transplanted)	
		264	0.23	453		(Not transplanted)	

* $\bar{X}$ ± SE given for all values except those of the sham-operated group for which data are given for each of the two mice that survived the 23-wk experiment.
† Two mice that each received two duct-ligated pancreata were still hyperglycemic (620, 468 mg/dl) at 23 wk and are included. Their pancreas contained 0.57, 0.56 μg IRI and 876, 971 ng IRG; their transplants contained 0.84, 0.85 μg IRI and <18, 327 ng IRG.

and IRG was reduced by two-thirds. In the two sham-operated mice that survived the experiment, pancreatic IRI was less than 2% of that in the nondiabetic pancreas. Pancreatic IRG was higher than normal in one animal and below normal in the second.

The IRI content in transplants plus that in the endogenous pancreas of isografted mice totaled 19% or 22% of the IRI contained in the nondiabetic pancreas. The total IRG content in transplant recipients totaled 54–60% of that in the nondiabetic pancreas.

Relationship between carbohydrate tolerance and content of pancreatic hormones in mice given incremental doses of streptozotocin (Table 4 and Figure 3). A graded depletion of pancreatic IRI content ranging from 64% to 3% of that in the normal mouse pancreas was observed in mice killed 23 wk after receiving incremental doses of STZ. In Table 4, data are presented in order of decreasing pancreatic IRI content, and are presented with the corresponding basal and meal-induced plasma glucose and IRI responses obtained in mice 2 or 3 wk before killing. Note that the basal fasting plasma glucose was slightly above the normal range (80–164 mg/dl, $\bar{X}$ ± 2 SD) if the IRI content fell to 14% of normal (~6μg) and highly elevated (>350 mg/dl) if the IRI content dropped to 4% (~2 μg). No consistent relationship between pancreatic IRI content and fasting plasma IRI was noted. As shown in Figure 3 and Table 4, plasma glucose of all STZ-treated mice 15 min after a meal was slightly above the $\bar{X}$ ± 2 SD of the plasma glucose in normal mice, and

TABLE 4
Plasma glucose (G) and immunoreactive insulin (IRI) after a 7-h fast or 15 min after a 15-min meal of mouse food* and pancreatic immunoreactive insulin (IRI) and glucagon (IRG) in mice 23 wk after receiving incremental doses of streptozotocin (STZ)

Rank† order	Dose of STZ (mg/kg)	Pancreatic IRI (μg)	Pancreatic IRI % of normal	Pancreatic IRG (ng)	Pancreatic IRG % of normal	Basal fasting Plasma G (mg/dl)	Basal fasting Plasma IRI (ng/ml)	15 min after a meal Plasma G (mg/dl)	15 min after a meal Plasma IRI (ng/ml)
(Normal)‡	0	43.2 ±20.0	100	1626 ±1022	100	122 ±42	0.26 ±40	126 ±34	1.59 ±1.32
1	140	27.6	64	1620	100	126	0.17	186	2.76
2	140	24.3	56	1161	71	119	0.35	148	1.91
3	140	20.4	47	930	57	89	0.36	194	3.16
4	140	20.3	47	1335	82	79	0.15	183	1.91
5	160	17.9	41	1338	82	133	0.24	174	2.26
6	180	17.8	41	1485	91	104	0.19	200	1.82
7	160	13.5	31	1293	80	140	0.50	172	2.85
8	200	12.6	29	990	61	129	1.18	255	2.30
9	180	11.6	27	948	58	103	0.84	165	2.12
10	180	11.1	26	1425	88	117	0.83	186	1.72
11	200	11.2	26	1440	88	76	1.10	259	1.22
12	200	10.1	23	1485	91	120	1.30	317	1.82
13	260	7.3	17	1695	104	153	0.90	389	1.05
14	260	5.9	14	2130	131	180	0.20	416	1.22
15	220	5.7	13	1575	97	174	0.56	347	0.81
16	220	5.0	12	2370	146	188	0.56	388	0.92
17	240	1.9	4	2160	133	377	0.29	611	0.49
18	240	1.3	3	1347	83	405	0.67	619	0.34

* Basal and meal-induced plasma glucose and IRI obtained 3 or 4 wk before mice were killed.
† Ranked in order of decreasing pancreatic IRI content.
‡ The $\bar{X}$ ± 2 SD, N = 13, are given for normal control mice not treated with streptozotocin.

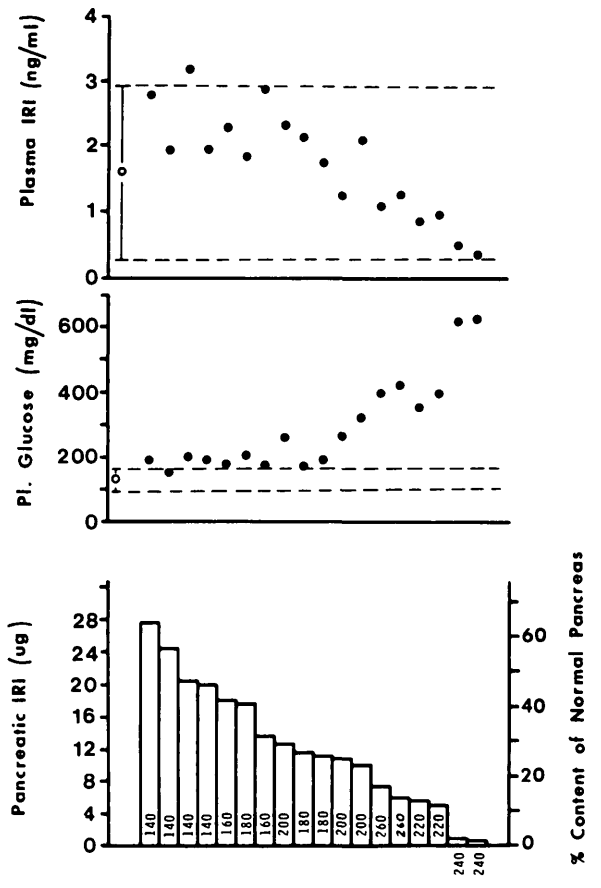

FIGURE 3. Pancreatic IRI content (presented in order of decreasing content) and plasma glucose and immunoreactive insulin (IRI) in mice given incremental doses of streptozotocin 15 min after a 15-min meal. Doses of STZ (mg/kg mouse) are given at base of bars. Dashed lines represent $\bar{X} \pm 2$ SD of plasma glucose and IRI of normal mice.

was substantially above this range in those mice whose pancreatic IRI content was 26% of the normal content or below. Though the plasma IRI responses after a meal tended to decline as pancreatic IRI content decreased, plasma IRI levels were still within the $\bar{X} \pm 2$ SD of that in normal mice.

No apparent relationship between carbohydrate tolerance and pancreatic IRG content was noted in streptozotocin-treated mice.

DISCUSSION

As observed in the present study, transplant recipients displayed impaired glucose tolerance and subnormal insulin responses after intravenous or gastric glucose challenges. After an overnight fast and a 15-min meal, isografted mice still showed severely impaired glucose tolerance, but their insulin responses, in several instances, were not significantly different from those of nondiabetic controls. In normal animals, hepatic extraction of insulin results in peripheral levels of the hormone that are one-half to one-third of those in the portal circulation.[6] In mice with subcutaneous islet transplants, insulin levels in the periphery are probably similar to those entering the portal vein. Thus, transplant recipients are still relatively insulin deficient at the site of the liver. This deficiency could result in a failure to stem glucose output or to initiate glucose uptake by the liver which, in turn, would lead to glucose intolerance.

Other disruptions occurred after heterotopic pancreatic transplantation that could have contributed to glucose intolerance. In particular, the normal parasympathetic and sympathic innervation of the islets was severed, and could have resulted in perturbation of the stimulatory-inhibitory neural reflex for insulin secretion.[7] Pipeleers et al.[8] observed that transplanted islets, like other denervated organs, displayed hypersensitivity to catecholamines. Indeed, the suppressed insulin responses to intravenous glucose that were observed in islet-transplanted rats under stress were similar to the insulin responses after i.v. glucose observed in our studies[1,2] and in those of others.[9–11] In adrenalectomized rats and in isografted animals tested for glucose tolerance under nonstressed conditions, as in rats injected via previously implanted catheters, peak insulin responses and tolerance to i.v. glucose were improved substantially[8,9] or were essentially normalized.[12,13]

Yet, even in nonstressed, isografted animals, an absence or delay of the early, preabsorptive peak of insulin secretion after an oral stimulus has been observed.[9,12] This abnormality has been attributed to the absence of vagally mediated stimulation of insulin secretion from specifically denervated, transplanted islets.[12,14] Our choice of sampling times precluded the characterization of the earliest phase of insulin secretion in our studies. Nevertheless, subcutaneous islet transplants lack the usual autonomic innervation, and if after further study the early insulin response is indeed lacking, this would help explain why glucose tolerance was impaired.

Notably, peak insulin responses of transplant recipients were greater after gastric glucose or a meal than after intravenous glucose, even though peripheral plasma glucose was just as high after the intravenous injection. Evidently, the insulinogenic factors ("incretin")[15] released by the gastrointestinal mucosa were responsible for the greater insulin response after gastric glucose or food ingestion.

Regarding glucose homeostasis after food ingestion, we noted that fasted transplant recipients consumed a smaller amount of food and showed lower postabsorptive plasma glucose after a 3-min meal than after a 15-min meal. Since mice fed ad libitum consume their food in small frequent meals, substantial deviations from normal glucose homeostasis probably rarely occurs. This may explain why plasma glucose levels have usually been within the normal range even when transplant recipients were in the nonfasted state.[1,2] Louis-Sylvestre has reported that feeding behavior does not change after rodents have recovered from experimental diabetes after islet transplantation.[16]

There is some evidence that the degree of glucose intolerance and the magnitude of peak insulin response are at least partially dependent on the insulin content or islet mass in transplants[17,18] or on the amount of islet tissue initially transplanted.[19,20] Although somewhat different from the transplant situation, studies in which the insulin content or islet mass were partially reduced by alloxan or streptozotocin injection, or by surgical removal, indicated that impaired glucose tolerance and subnormal insulin responses resulted from a reduction of insulin content or islet mass

below 50–60% of normal.[21–23] In streptozotocin-treated mice of the strain used in our studies, impaired glucose tolerance after food ingestion and insulin responses in the lower range of normal were noted if pancreatic insulin content was reduced below 26% of the normal content. By comparison, mice transplanted with either two or five duct-ligated pancreata both showed the same degree of glucose intolerance and subnormal insulin responses. Despite the initial transplantation of different amounts of islet tissue, insulin content in transplant recipients (pancreas plus transplant) at 23 wk was essentially alike, totaling 19–22% of that in the pancreas of age-matched controls. Although a higher insulin content in transplant recipients might show an effect, we have failed to show any relationship between glucose tolerance or insulin responses and pancreatic hormone stores.

On the other hand, relationships between the incidence or rate of recovery from hyperglycemia and the amount of islet tissue initially transplanted,[4] or the insulin content of transplants after transplantation,[1,3] seem to be well established and have been confirmed by others.[17,19] These relationships indicate that transplant insulin stores are substantially greater in mice that recover from hyperglycemia than in mice that fail to recover.[1,3] Also, the rate of recovery is inversely correlated with the amount of islet tissue initially transplanted[4] or the insulin content in transplants.[1]

Because of these relationships, changes in hormone storage capacity of pancreatic islet tissue after transplantation are important. Although the data are sketchy and must be viewed rectrospectively from several experiments,[1,2] we have some idea of these temporal changes. During the revascularization phase after transplantation, a substantial reduction of transplant hormone stores occurred amounting to 80% of those initially transplanted.[1] The loss of insulin stores was nearly proportional to the loss of glucagon. Between 7 and 18 wk, insulin content increased but glucagon content remained unchanged.[2] In the present study, insulin content was essentially the same for transplants of either two or five duct-ligated pancreata at 23 wk, but glucagon content was less in the transplants of two duct-ligated pancreata. Our interpretation of the temporal changes in the content of hormones in these transplants is shown in Figure 4. We assumed that 80% of the hormone stores initially transplanted were lost within 6 wk. Since mice transplanted with two duct-ligated pancreata took longer to recover, we suspect that the insulin content was less in these transplants at 6 wk, but increased thereafter, so that at 23 wk the insulin content was the same as that in transplants of five duct-ligated pancreata. Since we have observed that glucagon content remains unchanged after recipients recover from hyperglycemia,[2] this would account for the lower glucagon content in two duct-ligated pancreata at 23 wk. This interpretation implies that the increase in transplant IRI stores may be limited.

Perhaps just as important as changes in hormone levels in transplants are those changes occurring in the recipients' endogenous pancreas. Earlier we reported that insulin content in pancreata of just recovered recipients, although low in relation to that in the normal pancreas, was significantly higher than that in pancreata of sham-operated mice.[1] Several weeks after the recipients' recovery, an additional significant increase in IRI content was noted.[2] In the present study, pancreatic insulin content was as much as sevenfold higher than that of severely hyperglycemic, sham-operated mice. This factor agrees with that observed by Trimble et al.[25]

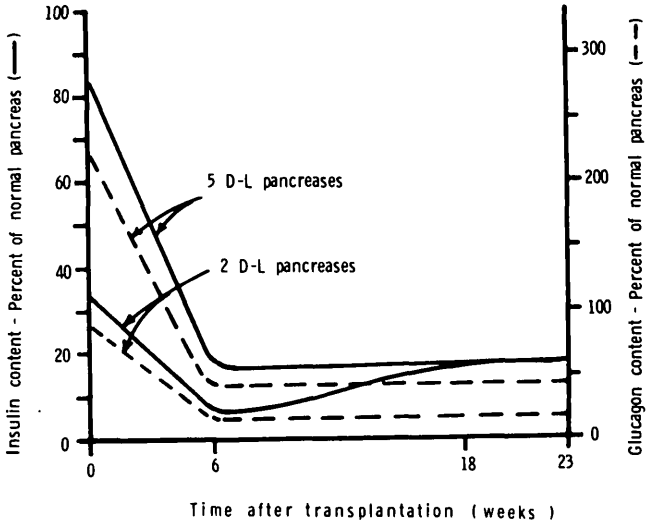

FIGURE 4. Temporal changes in immunoreactive insulin (IRI) and glucagon (IRG) content in transplants of two or five duct-ligated pancreata after subcutaneous, isogeneic transplantation in streptozotocin-diabetic mice. An interpretation based on data of present and past reports.[1,2]

Glucagon content in the endogenous pancreas displayed even more substantial changes than that of insulin. As reported by ourselves[1,2] and others,[26] the highly elevated glucagon levels in the pancreas of streptozotocin-treated rodents decrease after recipients recover from hyperglycemia. In the present study, the glucagon content in the pancreas of transplant recipients was only one-third of that in the pancreas of age-matched controls. Thus, the substantial reduction of glucagon stores in the endogenous pancreas and the small but significant increase in insulin stores may contribute to the recipients' recovery from hyperglycemia.

ACKNOWLEDGMENTS

We are most grateful for the technical assistance of Linda M. Kramp, and we also thank Linda M. Kramp and June Almond for typing the manuscript.

This work was supported in part by the Vanderbilt University Diabetes-Endocrinology Research Center, Grant AM 17026, and in part from Biomedical Research Support Grant 2-507-RR 07095-12 to Virginia Polytechnic Institute and State University.

REFERENCES

[1] Kramp, R. C., Cuche, R., Muller, W. A., and Renold, A. E.: Subcutaneous, isogeneic transplantation of duct-ligated pancreas in streptozotocin diabetic mice: relationships between recovery and hormone contents in transplants or host pancreas. Metabolism *25*:1007–15, 1976.

[2] Kramp, R. C., and Renold, A. E.: Subcutaneous, isogeneic transplantation of duct-ligated pancreas in streptozotocin diabetic mice II. Hormone storage as a function of time and of the recipient's initial glycemic state. Metabolism *30*:644–48, 1981.

[3] Kramp, R. C., Cuche, R., and Renold, A. E.: Subcutaneous, isogenic transplantation of either duct-ligated pancreas or isolated islets in streptozotocin diabetic mice. Endocrinology *99*:1161–67, 1976.

[4] Kramp, R. C., Congdon, C. C., and Smith, L. H.: Isogenic or alloge-

neic transplantation of duct-ligated pancreas in streptozotocin diabetic mice. Eur. J. Clin. Invest. *5*:249–58, 1975.

[5] Herbert, V., Lau, K. S., Gottlieb, C. W., and Bleicher, S. J.: Coated charcoal immunoassay of insulin. J. Clin. Endocrinol. Metab. *25*:1375–84, 1965.

[6] Field, J. B., Rojdmark, S., Harding, P., Ishida, T., and Chou, M. C. Y.: Role of liver in insulin physiology. Diabetes Care *3*:255–60, 1980.

[7] Woods, S. C., and Porte, D., Jr.: Neural control of the endocrine pancreas. Physiol. Rev. *54*:596–619, 1974.

[8] Pipeleers, D. G., Pipeleers-Marichal, M. A., Karl, I. E., and Kipnis, D. M.: Secretory capability of islets transplanted intraportally in the diabetic rat. Diabetes *27*:817–24, 1978.

[9] Trimble, E. R., Siegel, E. G., Berthoud, H.-R., and Renold, A. E.: Intraportal islet transplantation: functional assessment in conscious unrestrained rats. Endocrinology *106*:791–97, 1980.

[10] Eloy, R., Kedinger, M., Garaud, J. C., Hafen, K., Launey, J. F., Moody, J., Clendinnen, G., and Grenier, A. F.: Intrahepatic transplantation of pancreatic islets in the rat. Horm. Metab. Res. *9*:40–46, 1977.

[11] Steffes, M. W., Sutherland, D. E. R., Mauer, M., Leonard, R. J., Najarian, J. S., and Brown, D. M.: Plasma insulin and glucose levels in diabetic rats prior to and following islet transplantation. J. Lab. Clin. Med. *85*:75–81, 1975.

[12] Louis-Sylvestre, J.: Relationship between two stages of prandial insulin release in rats. Am. J. Physiol. *235* (2):E103–11, 1978.

[13] Brown, J., Clark, W. R., Molnar, I. G., and Mullen, Y. S.: Fetal pancreas transplantation for reversal of streptozotocin-induced diabetes in rats. Diabetes *25*:56–64, 1976.

[14] Jeanrenaud, B., Berthoud, R.-R., Bereiter, D. A., Rohner-Jeanrenaud, F., and Hochstrasser, A.-C.: Ventromedial ventrolateral hypothalamus and other CNS sites and their relationships with the activity of the endocrine pancreas. *In* Diabetes 1979. Waldhausl, W. K., Ed. Amsterdam, Excerpta Medica, International Congress Series No. 500, 1980, pp. 51–56.

[15] Creutzfeldt, W.: The incretin concept today. Diabetologia *16*:75–85, 1979.

[16] Louis-Sylvestre, J.: Relationship between two stages of prandial insulin release in rats. Am. J. Physiol. *235* (2):E119–25, 1978.

[17] McEvoy, R. C., Schmitt, R. V., and Hegre, O. D.: Syngeneic transplantation of fetal rat pancreas I. Effects of insulin treatment on the reversal of alloxan diabetes. Diabetes *27*:982–87, 1978.

[18] McEvoy, R. C., and Hegre, O. D.: Syngeneic transplantation of fetal pancreas III. Effect of insulin treatment on the growth and differentiation of the pancreatic implants after reversal of diabetes. Diabetes *28*:141–46, 1979.

[19] Leonard, R. J., McEvoy, R. C., Schmitt, R. V., Lazarow, A., and Hegre, O. D.: Intraperitoneal isotransplantation of enzymatically dispersed pancreas: effects on donor islet mass and donor age on reversal of alloxan diabetes. *In* Proceedings of the Ninth Congress of the International Diabetes Federation. Bajaj, J. S., Ed. Amsterdam, Excerpta Medica, 1977, pp. 544–51.

[20] Panijayanond, P., Soroff, H. S., and Monaco, A. P.: Pancreatic islet isografts in mice. Surg. Forum *24*:329–31, 1973.

[21] Pupo, A. A., Ursick, M. J. M., Iamaguchi, E., and Vasconcellos, F. G.: Acute- and late-phase of insulin secretion and glucose tolerance in mild alloxan diabetes in dogs. Diabetes *25*:161–66, 1976.

[22] Sun, A. M., Coddling, J. A., and Haist, R. E.: A study of glucose tolerance and insulin response in partially depancreatized dogs. Diabetes *23*: 424–32, 1974.

[23] Junod, A., Lambert, A. E., Stauffacher, W., and Renold, A. E.: Diabetogenic action of streptozotocin: relationship of dose to metabolic response. J. Clin. Invest. *48*:2129–39, 1969.

[24] Hegre, O. D., Leonard, R. J., Schmitt, B. A., and Lazarow, A.: Isotransplantation of organ-cultured neonatal pancreas: reversal of alloxan diabetes in the rat. Diabetes *25*:180–89, 1976.

[25] Trimble, E. R., Malaisse-Lagae, F., and Vassutine, I.: Effects of transplantation on the recipient pancreas and on the transplanted pancreatic islets. Diabetes *27* (2):440, 1978. Abstract.

[26] Pipeleers-Marichal, M. A., Pipeleers, D. G., Cutler, J., Lacy, P. E., and Kipnis, D. M.: Metabolic and morphologic studies in intraportal-islet-transplanted rats. Diabetes *25*:1041–51, 1976.