Normalization of Six Key Hepatic Enzymes After Fetal Pancreas Transplantation in Diabetic Rats

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SUMMARY

In diabetic rats transplanted with fetal pancreata we measured the activities of six important enzymes to assess the return of liver metabolism to normal. Comparison was made among the responses of transplanted rats with and without renal-portal vein shunts and of those not transplanted and injected with insulin in varying doses. Insulin supply was not limited since three or four fetal pancreata were first grown in normal rats before transfer into the diabetic animals.

Transplantation normalized blood and urine glucose and the rate of disappearance of intravenous glucose. Glucokinase and pyruvate kinase activities in liver rose toward normal at 7 days after transplantation and reached normal levels at 30 and 90 days. The response of the other four enzymes, glucose-6-phosphate dehydrogenase, citric lyase, fructose-1,6-bisphosphatase, and glucose-6-phosphatase, was more rapidly restored to normal at 7 days and remained normal at 30 and 90 days. No difference was observed in the enzyme activities of transplanted-shunted rats to nonshunted animals.

Glucokinase activity was restored to normal after 1 wk of daily injections of 1 U of PZI; pyruvate kinase restoration required 3 U/day. Glucose-6-phosphate dehydrogenase and citric lyase required 2 U/day to be restored to normal; 3 U daily resulted in temporary supernormal activities. The gluconeogenic enzymes, fructose-1,6-bisphosphatase and glucose-6-phosphatase, were only partially suppressed toward normal by insulin even with 3 U daily for 3 wk.

These findings indicate that pancreas transplantation is a more effective regulator of liver metabolism in diabetes than insulin injections. DIABETES *32*:730–733, August 1983.

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An alternative method to injections for replacing insulin in diabetes is pancreatic transplantation. We have demonstrated in rats made diabetic with streptozotocin that transplantation of syngeneic fetal pancreata can return blood glucose and intravenous glucose tolerance to normal.[1]

Evidence that the transplant delivers insulin in a normal homeostatic manner, in addition to normal blood glucose and insulin levels, comes from the observation that transplanted female rats maintain normal blood glucose levels throughout pregnancy.[2] In addition, blood glucagon levels of transplanted male rats are normal, both in the basal state and in response to arginine stimulation.[3]

Plasma insulin levels are mildly elevated above normal in transplanted rats, which is probably due to the venous drainage from the grafts beneath the kidney capsule into the renal vein bypassing the liver. Construction of a renal-portal vein shunt to deliver the secreted insulin directly to the liver greatly augments the effects of transplantation and lowers peripheral insulin levels to normal.[4]

The liver is a primary site for insulin action in regulating metabolism.[5,6] To determine whether or not transplantation of fetal pancreata more effectively regulates metabolism than insulin injections, we measured the activity of six important enzymes in the liver. Comparisons were made among the responses of transplanted rats with and without renal-portal vein shunts and of those untransplanted and injected with insulin in varying doses.

MATERIAL AND METHODS

Animals. Lewis male rats, 250–300 g, purchased from Charles River Breeding Laboratories (Wilmington, Massachusetts) for insulin studies or from Microbiological Associates (Walkersville, Maryland) for transplant studies, were fed ad lib on Purina chow and housed in a room with 12 h of darkness and 12 h of light. During the metabolic studies, rats were kept in metabolic cages and urine glucose was

TABLE 1
Blood glucose concentration and urine volume and glucose over a 24-h period

Experimental group	N	Treatment	Blood glucose (mg/dl)			Urine	
			9 a.m.	3 p.m.	9 p.m.	ml/24 h	g/24 h
Normal control	8	None	120 ± 4*	120 ± 5	117 ± 4	13 ± 2	0
Diabetic control	15	None	507 ± 28	—	—	92 ± 5	6.4 ± 0.5
Insulin-treated diabetic	7	1 PZI 3×/day	213 ± 27	111 ± 42	189 ± 16	20 ± 0.3	0.5 ± 0.1
	3	3 PZI 1×/day	352 ± 49	80 ± 10	98 ± 6	—	—
Transplanted diabetic	8	Fetal pancreas transplant	130 ± 4	132 ± 3	135 ± 4	12 ± 1	0

*Mean ± SEM.

determined 3 times per week from a 24-h urine collection. Blood was collected from the tail vein just before insulin injection, and glucose content was measured on a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, California). Normal and diabetic control groups consisted of approximately equal numbers from two suppliers; no metabolic differences were observed.

Induction of diabetes. Diabetes mellitus was induced by injecting, into a tail vein, streptozotocin (65–70 mg/g) dissolved in citrate buffer, pH 4.5. Control animals were injected with a similar volume of citrate buffer only. Animals were fasted for 12 h, after which they underwent glucose tolerance tests by intravenous injection of 0.5 g/kg body wt of glucose. Tail vein blood samples were then obtained at 10, 20, 30, and 40 min.

Transplantation procedures. Procedures for transplantation have been reported previously.[1] Fetal pancreata (three or four) were placed beneath the kidney capsules of normal syngeneic adult animals and grown for 3 wk. Then, the kidneys along with the transplanted pancreata were transferred to diabetic recipients, which were monitored for a minimum of 3 mo. At the time of transplantation, some of the diabetic animals had the renal vein of the transplanted kidney diverted to the hepatic portal vein.[4] No transplanted animal received insulin at any time.

Insulin therapy. Insulin was dissolved in phosphate-buffered saline (10 U of PZI/ml) and injected subcutaneously once (9 a.m.), twice (9 a.m. and 10 p.m.), or 3 times per day at 9 a.m., 3 p.m., and 10 p.m. to give a daily dose of 1, 2, or 3 U. The animals that received 3 U one time per day received the insulin at 9 a.m. Controls were injected with a corresponding volume of phosphate-buffered saline.

Liver samples. Transplanted diabetic animals were anesthetized, and approximately 1 g of liver tissue was removed from the large lobe before and following transplantation for enzyme assay. After 3 mo the transplant was removed and recurrence of diabetes monitored. Data were not used from animals that had no recurrence of diabetes after removal of the transplant. Insulin-treated and untreated diabetic animals were killed by guillotine and 1-g portions of liver tissue from the large lobe were removed for analysis. Diabetic controls were used 1–3 wk after streptozotocin injection; liver enzyme activities were stable during this interval.

Tissue preparation. One portion of liver tissue was immediately frozen to −70°C for later determination of glucose-6-phosphatase. The other portion was minced on ice and homogenized in 5 vol of cold buffer: 150 mM KCl, 5 mM $MgCl_2$, 5 mM EDTA, and 10 mM beta-mercaptoethanol. The homogenate was centrifuged in a Beckman high-speed centrifuge at 100,000 × *g* for 45 min at 4°C. One portion of the supernatant was used for the glucokinase assay after being dialyzed for 1–2 h against homogenization buffer (1:25) to remove glucose. The remainder was used fresh for enzyme analyses and protein determinations.

Enzyme analyses. Enzymes were assayed by modified standard methods at two different concentrations to assure proportionality. A Beckman 25 spectrophotometer with continuous records at 340 mμm at 25°C was used to measure the conversion of NADP to NADPH in the presence of pyruvate kinase,[7] fructose-1,6-bis-phosphatase,[8] ATP-citric lyase,[9] glucose-6-phosphate dehydrogenase,[10] and glucokinase/hexokinase.[11] Glucose-6-phosphatase was measured[12] by the release of inorganic phosphate[13] and expressed as phosphate released per minute at 37°C/g protein[14] in the liver homogenate. Enzyme activities were expressed as the percentage of activity in the livers of 15 normal control animals.

TABLE 2
Effect of 65–70 mg/kg streptozotocin on hepatic enzymes

Enzyme	Activity (U/g supernatant protein)		
	Control (N = 12)	Diabetic (N = 12)	P
Glucokinase	11.1 ± 1.0*	Neg. to 1.8	
Pyruvate kinase	386 ± 40	161 ± 32	< 0.001
Glucose-6-phosphate dehydrogenase	32.7 ± 4.0	20.4 ± 2.2	< 0.02
ATP-citric lyase	21.5 ± 1.1	5.0 ± 1.1	< 0.001
Fructose-1,6-bis-phosphatase	39.8 ± 4.6	62.4 ± 10.4	< 0.1
Glucose-6-phosphatase	66.0 ± 5.0	139 ± 15.8	< 0.001

*Mean ± SEM.

RESULTS

Control of diabetes. Based on the usual criteria, diabetes was completely reversed following pancreas transplantation in both the nonshunted and shunted groups of rats (Table 1). Blood glucose measured at 9 a.m., 3 p.m., and 9 p.m.

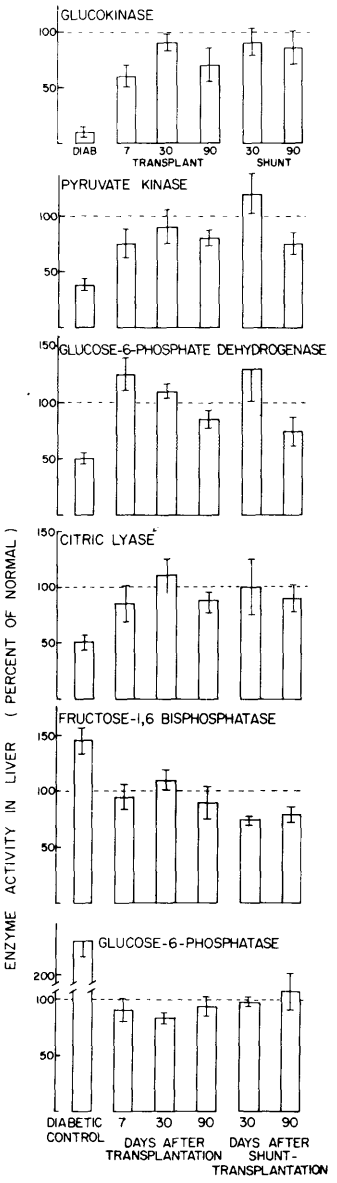

FIGURE 1. Enzymatic activities in the livers of streptozotocin-diabetic rats before and at intervals after transplantation of 3–4 pregrown fetal pancreata contained beneath the renal capsule of a normal syngeneic rat. Comparison is made to the enzyme activities in the livers of rats in which renal vein-portal vein shunts were done at the time of transplantation of the kidney and pancreas. Mean ± SEM.

was normal; glucose disappeared from the urine and urine volume returned to normal. Additionally, the disappearance rate of intravenous glucose from the blood in transplanted rats with and without renal-portal vein shunts was no different from that of normal controls.

In contrast, nontransplanted diabetic rats injected with 1 U of insulin (PZI) 3 times daily were hyperglycemic during part of the day. If the insulin was given in one dose of 3 U at 9 a.m., the morning blood sugar was even higher (Table 1). Urine glucose fell to 0.5 ± 0.2 g/day in rats given three injections daily compared with the 6.4 ± 2.3 g/day excreted by diabetic controls.

Effect of streptozotocin diabetes on hepatic enzyme activities (Table 2). The activities of the glycolytic enzymes glucokinase and pyruvate kinase fell to less than one-half of normal in the livers of diabetic rats. There was a similar fall in the enzymes involved in lipid synthesis, glucose-6-phosphate dehydrogenase, and citric lyase, in contrast with the gluconeogenic enzymes fructose-1,6-bisphosphatase and glucose-6-phosphatase, which were nearly double the normal value. No significant difference was observed in the weight of the livers of diabetic rats (8.9 ± 0.5 g) when compared with normal controls (9.9 ± 0.6 g).

Effect of pancreas transplantation on hepatic enzymes (Figure 1). Glucokinase and pyruvate kinase activities were partially restored toward normal at 7 days after transplantation and reached a normal level when sampled at 30 and 90 days. The responses of the other four enzymes, glucose-6-phosphate dehydrogenase, citric lyase, fructose-1,6-bisphosphatase, and glucose-6-phosphatase, were more rapidly restored to normal levels at 7 days after transplantation and remained normal at 30 and 90 days. The enzyme activities in the livers of transplanted-shunted rats, when measured at 30 and 90 days after transplantation, were not different from nonshunted animals.

Effect of insulin injections on hepatic enzymes (Table 3). Glucokinase activity in the liver of diabetic rats is very sensitive to insulin injections and is restored to normal after 1 wk of daily subcutaneous injections of 1 U of PZI. The activity is the same after 2 or 3 U administered daily whether injected at separate times or at one time and whether injected for 1 or for 3 wk.

Pyruvate kinase is less sensitive to insulin and is restored to normal only by the administration of 3 U of insulin per day, whether injected in three 1-U doses or one 3-U dose. The enzymes mediating lipid synthesis, glucose-6-phosphate dehydrogenase and citric lyase, require 2 U/day to be restored to normal; 3 U daily results in supernormal activities. This hyperstimulation is temporary, and after 3 wk of insulin given in 1 U three times daily, the activities of both enzymes return to normal.

The gluconeogenic enzymes, fructose-1,6-bisphosphatase and glucose-6-phosphatase, are only partially suppressed toward normal, even with 3 U of insulin per day and even when given for 3 wk. The only exception is that after 1 wk of insulin in a dose of 1 U three times daily, fructose-1,6-bisphosphatase activity is normal.

DISCUSSION

There has been an increasing acceptance of a relationship between the control of blood sugar in diabetes and the development of vascular complications. This has led to the prescription of multiple daily insulin injections or the use of a mechanical device to deliver insulin in a manner simulating the normal pancreas.

Our contention from previous studies is that following fetal pancreas transplantation, insulin is released in a homeostatic fashion in accordance with metabolic requirements. In this study we measured the activity of six important hepatic enzymes in the livers of untreated diabetic rats at intervals of up to 90 days following transplantation of fetal pancreata. We discovered that the activities of these enzymes returned to normal following transplantation of fetal pancreata into adult diabetic rats, thereby providing additional support for our former findings.

We were initially surprised that there is no difference in

TABLE 3
Effect of subcutaneous insulin injections on enzyme activity in the livers of diabetic rats

Treatment	N	Glucokinase	Pyruvate kinase	Glucose-6-phosphate dehydrogenase	Citric lyase	Fructose-1,6-bis-phosphatase	Glucose-6-phosphatase
		(Percent of enzyme activities in livers of normal rats)					
Diabetic control	8	10 ± 15*	42 ± 5	62 ± 5	23 ± 6	157 ± 12	211 ± 13
Insulin (PZI) 7 days							
1 U/day	6	107 ± 20	60 ± 18	73 ± 14	88 ± 16	149 ± 22	143 ± 7
1 U 2×/day	3	115 ± 3	64 ± 5	83 ± 8	124 ± 1	135 ± 10	149 ± 17
1 U 3×/day	7	115 ± 5	123 ± 11	144 ± 18	163 ± 6	102 ± 6	131 ± 7
3 U/day	7	100 ± 8	137 ± 11	146 ± 13	173 ± 8	136 ± 5	118 ± 7
Insulin (PZI) 21 days							
1 U 3×/day	8	86 ± 8	94 ± 7	84 ± 9	88 ± 7	120 ± 8	128 ± 6

*Mean ± SEM.

liver enzyme activities between the shunted rats, in which insulin is delivered directly into the liver, and nonshunted animals, in which insulin is delivered into the general circulation. In previous experiments in which the insulin supply was limited, the shunt operation dramatically improved control of glucose metabolism.[4] In the experiments reported here, however, the insulin supply is not limited since three or four fetal pancreata were first grown in normal carrier rats before being transferred into the diabetic animals to avoid having to administer insulin injections during the initial growth period. Moreover, comparisons between shunted and nonshunted rats were made only 30 days after transplantation; it is possible that observations at an earlier stage posttransplantation might have indeed revealed a faster response in shunted animals.

In contrast to the transplanted diabetic animals, even the most vigorous schedule of insulin injection (1 U given 3 times daily) did not return blood or urine sugar completely to normal. Residual abnormalities in glucose control were accompanied by continued abnormalities in at least some liver enzyme activities. Insulin therapy was much less effective than fetal pancreas transplantation in suppression of overstimulated gluconeogenic enzymes, and in general the activities of these enzymes remained above normal. In spite of these findings, there was temporary overstimulation of lipogenic enzymes, but by 3 wk the activities returned to normal, confirming previous observations by others.[15]

The observation that liver enzyme activities in diabetic rats rapidly return to normal following transplantation of fetal pancreata suggests that this method more effectively regulates metabolism than does that of insulin injections.

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