Glucose Turnover and Insulin Sensitivity in Rats With Pancreatic Islet Transplants

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To study the metabolic effects of insulin derived from islet grafts, oral glucose tolerance (OGT) and glucose turnover were examined in streptozotocin-induced diabetic Lewis rats rendered normoglycemic by syngeneic islet grafts in the renal subcapsular space (REN), in REN with renal vein–to–mesenteric vein anastomosis (REN-RMA), in the liver (intrahepatic [IH]), or in a parahepatic omental pouch (POP) and compared with normal rats. Normal OGT was found at 1 month posttransplant in all animals receiving ~3,000 islets, with hyperinsulinemic responses in the REN group compared with the other groups, and with higher C-peptide responses in the IH group than in the other groups (P < 0.05 by one-way analysis of variance). Glucose turnover studies in the insulin-stimulated steady state (INS-SS; infusion of insulin at 10 pmol · kg^{-1} · min^{-1}) at 2 months posttransplant showed that whole body glucose disappearance rates (R_d) were similar in all groups, but the REN group had higher steady-state insulin levels than the other groups. Glucose infusion rates (GIRs) were lower in the REN and IH groups than in the other groups. Apparent endogenous glucose production (EGP) was not completely inhibited in the REN and IH groups, while complete inhibition was observed in the other groups. When INS-SS insulin levels were matched to the level in REN rats by increasing the insulin infusion rate to 20 pmol · kg^{-1} · min^{-1} in REN-RMA, IH, and normal rats, GIR and R_d were elevated, exceeding those values in REN rats, but GIR in IH rats was still lower than in REN-RMA and normal rats. Thus, *1*) in the REN group, impairment of inhibition of EGP and of stimulation of R_d by exogenous insulin contribute to insulin resistance; *2*) in the IH group, incomplete inhibition of EGP is the major determinant of insulin resistance; and *3*) with portal delivery of insulin in the REN-RMA and POP groups, normal insulin sensitivity is preserved. The present study confirms that hepatic portal delivery of islet secretions is necessary for physiological regulation of glucose metabolism. The study also suggests the IH grafts do not provide physiological regulation of glucose metabolism, raising the question of whether the liver is an appropriate site for insulin-secreting tissue replacement therapy in diabetes. *Diabetes* 47:1020–1026, 1998

Although apparent physiological advantages of hepatic-portal delivery of insulin over systemic delivery of the hormone have been suggested in several studies, both with respect to hepatic uptake of nutrients and the hormone, and with respect to the whole-body efficacy of insulin, their importance and mechanisms are not well understood. Various chronic animal models involving venous diversion of pancreatic islet endocrine secretions, with or without transplantation, have been used to address these questions. After complete diversion of the portal blood to the systemic circulation, hyperinsulinemia occurred in dogs with end-to-side portal-caval anastomosis (1), and hyperinsulinemia with insulin resistance occurred after portal-caval transposition in rats (2). However, decreased liver weight and liver blood flow were associated with portal-caval anastomosis, and systemic delivery of nutrients and gastrointestinal hormones confounded interpretation with portal-caval transposition. With pancreas or islet transplantation in animals with diabetes, the routes of insulin delivery can be varied, but the results among previous studies have been inconsistent with respect to associations of hyperinsulinemia and insulin resistance with particular routes (3–8). Hyperinsulinemia after a glucose challenge in dogs with segmental pancreas autografts with systemic drainage was present in one study (3), but not in another (4). Insulin resistance was found in dogs after pancreatic vein-to-inferior vena cava anastomosis (5), but corresponding findings after syngeneic islet transplantations in rodents have been variable, possibly due to different degrees of correction of glucose tolerance (6–8). Moreover, few of these animal studies documented the blood levels of C-peptide in the assessment of insulin secretion. In humans with pancreas transplantation and systemic venous drainage, hyperinsulinemia with elevated blood levels of C-peptide and insulin resistance has been shown (9,10), and hyperinsulinemia was not present with hepatic portal pancreas venous drainage (10). One of these studies also indicated that the liver plays a major role in insulin resistance after chronic systemic delivery of insulin in type 1 diabetic patients after pancreas transplantation (9). The discrepancies between the results of different studies may be due to variations among preparations and protocols.

We previously studied oral glucose tolerance (OGT) and insulin sensitivity in rats with renal subcapsular islet grafts (RENs) compared with normal rats (11). REN rats were also

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ANOVA, analysis of variance; AUC, area under the curve; EGP, endogenous glucose production; GIR, glucose infusion rate; IEQ, islet equivalent; IH, intrahepatic islet graft; INS-SS, insulin-stimulated steady state; MCR, metabolic clearance rate; OGT, oral glucose tolerance; OGTT, oral glucose tolerance test; POP, parahepatic omental pouch islet graft; R_d, rate of glucose disappearance; REN, renal subcapsular islet graft; RMA, renal vein–to–mesenteric vein anastomosis; STZ, streptozotocin.

compared with rats with renal subcapsular islet grafts and hepatic-portal diversion of secretions by renal vein–to–mesenteric vein anastomosis (RMAs) (11). Hyperinsulinemia with whole body insulin resistance, with decreased metabolic clearance of both endogenous (graft-derived) and exogenous insulin, was associated with chronic systemic delivery of insulin in REN rats, but findings in REN-RMA rats were normal. In the present study, we compared tracer-determined glucose turnover in REN rats to turnover in REN-RMA and normal rats. Moreover, since corresponding features in rats with intrahepatic islet grafts (IHs), and the possible importance of the tissue sites of grafts, are not clear, we conducted similar studies in IH rats, as well as in rats with presumed hepatic portal delivery of secretions from parahepatic omental pouch islet grafts (POPs).

RESEARCH DESIGN AND METHODS

Animal care. Male syngeneic Lewis rats (280–300 g; Harlan Sprague Dawley, Indianapolis, IN) were kept in an 0700–1900 light-dark cycle, with free access to rat diet (Prolab 3000; Agway, Syracuse, NY) and tap water. All procedures were approved by the animal care committee of the University of Western Ontario.

Research protocol. Animals were randomly allocated into five groups: REN, REN-RMA, IH, POP, and normal rats (Fig. 1). RMAs were performed 2 weeks before islet transplantation. Sham operations were performed in sets of REN (n = 8) and normal rats (n = 7) and did not affect the experimental results. At 1 week before islet transplantation, rats in the REN, REN-RMA, POP, and IH groups were given intravenous injections of streptozotocin (STZ) (85 mg/kg body wt; Sigma, St. Louis, MO). Diabetes, with polyuria, polydipsia, and weight loss, was confirmed by hyperglycemia. Before islet transplantation, animals were treated with NPH insulin injections (Iletin II, porcine insulin; Lilly, Indianapolis, IN) according to the tail blood glucose levels (OneTouch glucose meter; LifeScan, Milpitas, CA), as previously described (11). After transplantation, insulin was given according to random daily blood glucose levels until euglycemia (blood glucose <6 mmol/l) ensued. Oral glucose tolerance tests (OGTTs) were conducted 1 month after islet transplantation, and glucose turnover studies 2 months after islet transplantation, on conscious minimally restrained animals, starting between 1000 and 1100, after 11- to 12-h food withdrawal. All rats showed a hematocrit >45% at 0 min. Normal rats had body weights comparable to those of transplanted animals. At 2 weeks after the glucose turnover experiments, grafts were removed by nephrectomy in the REN (n = 6) and REN-RMA (n = 6) groups and by hepatolobectomy in the POP (n = 6) group, and tail vein blood glucose levels were monitored for 1 week. The removed kidneys and liver lobes bearing islet grafts were used for histology. In the rest of the animals, grafted tissues and pancreases were obtained at killing for histology.

RMA. Food was withdrawn 8–12 h before surgery under halothane (Wyeth-Ayerst, Montreal, Canada) anesthesia. After a midline incision, the left renal and superior mesenteric veins were exposed and isolated (Fig. 1). Then the renal vein was cross-clamped close to the kidney and ligated at the junction with the inferior vena cava. After dividing the renal vein close to the inferior vena cava, the superior mesenteric vein was clamped, and after venectomy, end-to-side renal vein–to–superior mesenteric vein anastomosis was performed with 10-0 nylon sutures. The clamping time was <15 min. In sham operations in REN and normal rats, the renal and superior mesenteric veins were clamped for the same time, without venectomy.

Islet isolation. Normal male Lewis rats (300–350 g) were used as donors. Islets were isolated by intra–bile duct collagenase digestion, followed by dextran gradient separation (Sigma MW 70,000; gradient composition: 27, 23, and 11%). After overnight culture, an aliquot of islets was counted as "islet equivalents" under a scaled microscope using diphenylthiocarbazone (Sigma) staining. One islet equivalent (IEQ) was the islet tissue mass equivalent to a spherical islet of 150 µm in diameter. The average numbers of IEQ per islet graft were REN: 3,040 ± 218; REN-RMA: 2,970 ± 197; IH: 2,920 ± 212; and POP: 3,050 ± 229.

Islet transplantation. Islet transplantations were conducted at laparotomy under halothane anesthesia. In renal subcapsular islet transplantation, islets were injected via PE50 tubing inserted into the space beneath the kidney capsule. In parahepatic omental pouch islet transplantation, a segment of the greater omentum was attached to the inferior surface of one liver lobe using 10-0 nylon sutures, and islets were injected via PE50 tubing into the pouch. In intrahepatic islet transplantation, islets were injected into the portal vein via a 23-gauge needle connected to PE50 tubing.

Metabolic studies: OGTTs. Silastic catheters were inserted into the right jugular and left femoral veins under pentobarbital anesthesia (11), and tests were performed on resumption of normal body weight gain at least 4 days after cannulation. At 5 min before the test, blood was drawn for the postabsorptive levels. At 0 min, 25% dextrose solution (1.5 g dextrose/kg body wt) was given by gavage. Blood samples were taken at 10, 20, 30, 45, 60, 90, and 120 min in heparinized syringes with saline replacement, collected in tubes containing NaF (Sigma), and held on ice before collection of plasma, which was stored at –20°C until hormone assays. The total volume of blood drawn during the experiment was ~2 ml.

Metabolic studies: glucose turnover. To assess the whole body glucose disappearance rate (R_d), experiments were performed according to the description by Kahn et al. (12). Briefly, after a priming dose of [3-^{3}H]glucose (4 µCi; Amersham, Buckinghamshire, U.K.; high-performance liquid chromatography purified ~99.4%) at –60 min, continuous infusion (0.4 µCi/min) of [3-^{3}H]glucose was conducted throughout the experiment. Plasma samples for determination of [3-^{3}H]glucose specific activities were obtained at –25, –15, and –5 min. The euglycemic hyperinsulinemic clamps were conducted from 0 to 180 min, with infusion of regular porcine insulin (U-100R; Lilly) into the femoral vein, as reported elsewhere (11). For determination of R_d, aliquots of the jugular vein plasma were precipitated with $Ba(OH)_2$ and $ZnSO_4$ and centrifuged. Plasma [3-^{3}H]glucose specific activity was determined by liquid scintillation counting of the protein-free supernatant after evaporation to dryness. Data for R_d and apparent endogenous glucose production (EGP) represent the mean values during the last 15 min of the basal level study and the last 30 min of the euglycemic hyperinsulinemic clamp study (the insulin-stimulated steady state [INS-SS]). R_d was calculated by dividing the [3-^{3}H]glucose infusion rate by the mean plasma [3-^{3}H]glucose specific activity during the steady state, and EGP was calculated as the difference between the tracer-derived R_d and the glucose infusion rate (GIR). The total volume of blood withdrawn was <3 ml.

Assays. Plasma glucose determinations were performed using a Beckman Glucose Analyzer II (Fullerton, CA) immediately after each experiment. Plasma immunoreactive insulin was determined by radioimmunoassay with a dextran-coated charcoal separation (13) using ^{125}I-labeled insulin (Amersham Radiochemical Center, Amersham, U.K.), rat insulin standard from Novo (Copenhagen, Denmark) for all postabsorptive values, human insulin standard for determination of steady-state insulin levels in glucose turnover studies, and insulin antibody from P. Wright (Cambridge, U.K.). Human and porcine insulin standard curves were virtually superimposable in this assay system. Rat plasma immunoreactive C-peptide was determined with a kit (Linco, St. Louis, MO) using rat C-peptide standard (14). Plasma immunoreactive glucagon was measured with ^{125}I-labeled glucagon (Cedarlane, London, Ontario) and antibody from R. H. Unger (Dallas, TX).

Estimation of metabolic clearance of insulin. The metabolic clearance rate (MCR) of endogenous insulin was assessed by using the ratio of plasma concentrations of C-peptide to insulin in the postabsorptive state and by using the ratio of the areas under the concentration curves (AUCs) of C-peptide to insulin (0–120 min) after an oral glucose challenge as indexes (11). The MCR of exogenous insulin was calculated by dividing the insulin infusion rate by the mean plasma level of insulin in the INS-SS during the glucose turnover experiments (15).

Histology. Liver, kidney graft, and pancreas samples were taken at killing from all animals, preserved in formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Statistical analysis. A one-way analysis of variance (ANOVA) was used to determine treatment effects. When significant interactions were present, between-group comparisons were made using Student-Newman-Keuls tests. All data are expressed as means ± SE.

RESULTS

General conditions. Hyperglycemia (17.5 ± 0.2 mmol/l) developed within 24 h of STZ administration in all animals. In this condition, plasma C-peptide levels were undetectable. After islet transplantation, rats were euglycemic, independent from exogenous insulin, with normal body weight gain within 1 week. Euglycemia was sustained during the full course of studies. Graft removal caused hyperglycemia and resumption of insulin dependence within 24 h in the REN, REN-RMA, and POP groups. Histology showed islets in the renal subcapsular space in the REN and REN-RMA groups and in the parahepatic omental pouch in the POP group, with extensive revascularization. A small number of the islets in the POP group migrated into the adjacent liver parenchyma. In the IH group, islets penetrated the portal venules and resided in the space between the portal triangles and liver parenchyma. Islets in the native pancreases showed similar wasted morphology in all groups.

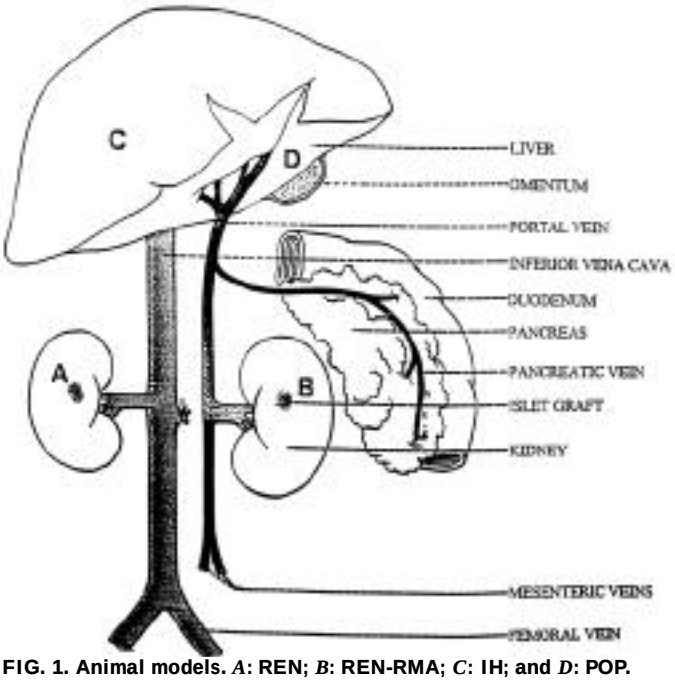

FIG. 1. Animal models. *A*: REN; *B*: REN-RMA; *C*: IH; and *D*: POP.

Postabsorptive state. Postabsorptive values for plasma glucose, insulin, C-peptide, glucagon, and C-peptide:insulin concentration ratios were obtained at 1 month (Table 1) and 2 months (Table 2) after islet transplantation. At 1 month, there were no significant differences in plasma glucose and glucagon levels among the groups. Insulin concentrations tended to be higher in REN rats, and the C-peptide:insulin ratio was significantly lower in REN rats than in REN-RMA, IH, POP, and normal rats. Peripheral plasma C-peptide levels and the C-peptide:insulin concentration ratios were significantly higher in IH rats than in REN, REN-RMA, POP, and normal rats. At 2 months after transplantation, there were no significant differences among plasma glucose, C-peptide, and glucagon levels, although C-peptide levels and the C-peptide:insulin concentration ratios were relatively high in IH rats. Insulin concentrations were significantly higher in REN rats than in REN-RMA, IH, POP, and normal rats, and the C-peptide:insulin ratio remained significantly lower in REN rats than in REN-RMA, IH, POP, and normal rats.

OGTTs. Plasma glucose responses were similar in all groups (Fig. 2 and Table 3). However, significantly higher insulin responses were observed in the REN group, with a significantly lower ratio of AUC (0–120 min) C-peptide to AUC insulin. Significantly higher C-peptide concentrations were observed in the IH group, with a higher ratio of AUC C-peptide to AUC insulin. No significant differences were found in the glucagon responses among groups.

Glucose turnover

Postabsorptive whole body* R_d *and EGP. R_d values in the postabsorptive state were similar in REN (n = 11, 7.0 ± 0.5 mg · kg^{-1} · min^{-1}), REN-RMA (n = 10, 7.9 ± 0.7), IH (n = 12, 7.3 ± 0.5), POP (n = 8, 7.6 ± 0.9), and normal rats (n = 10, 7.1 ± 0.8). In this state, these values also represented EGP.

INS-SS with an insulin infusion rate of 10 pmol · kg^{-1} · min^{-1}. During the INS-SS, R_d, plasma glucose, and glucagon levels were similar, while C-peptide levels fell to the assay detection limit (100 pmol/l for plasma samples) (Fig. 3 and Table 4). Maximal reductions of plasma [3-^{3}H]glucose specific activities (compared with basal values) were not different among groups. Plasma insulin concentrations were significantly higher in REN than in REN-RMA, IH, POP, and normal rats, while the tracer-derived values for R_d were similar among all groups. GIRs were normal in REN-RMA and POP rats and were significantly reduced in REN and IH rats. Thus, the apparent EGPs were significantly higher in REN and IH rats than in REN-RMA, POP, and normal rats.

Comparison of REN, REN-RMA, and IH groups with matching INS-SS insulin levels. To make valid comparisons of insulin sensitivity and glucose turnover, the insulin infusion rate was increased from 10 to 20 pmol · kg^{-1} · min^{-1} in REN-RMA, IH, and normal rats, matching the mean INS-SS plasma insulin level in REN rats. During the INS-SS, plasma glucose and glucagon levels were similar, while plasma C-peptide levels were decreased to the assay detection limit (Fig. 3 and Table 5). Maximal reductions of specific activities were significantly greater in REN-RMA (27 ± 7%), IH (36 ± 3%), and normal (48 ± 8%) rats than in REN (2 ± 12%) rats, $P < 0.04$. GIRs and values for R_d were increased in REN-RMA, IH, and normal rats compared with the respective values during infusion of 10 pmol · kg^{-1} · min^{-1} insulin and were significantly higher than those in REN rats. However, GIR in the IH group was still lower than GIR in the REN-RMA and normal groups, while the mean apparent EGP in the IH group was lower than EGP in the same group during infusion of 10 pmol · kg^{-1} · min^{-1} insulin and lower than that in the REN group but higher than that in the REN-RMA and normal groups.

DISCUSSION

This study amplifies our earlier comparisons of islet secretary

TABLE 1
Postabsorptive levels of plasma glucose, insulin, C-peptide, glucagon, and C-peptide:insulin ratios (CPIR) at 1 month posttransplant

	REN rats	REN-RMA rats	IH rats	POP rats	Normal rats	P
n	12	8	12	13	8	
Glucose (mmol/l)	6.0 ± 0.1	5.8 ± 0.2	6.1 ± 0.2	6.0 ± 0.1	5.9 ± 0.2	NS
Insulin (pmol/l)	100 ± 17	69 ± 8	67 ± 9	66 ± 11	71 ± 5	NS
C-peptide (pmol/l)	205 ± 12	226 ± 23	344 ± 27*	220 ± 21	240 ± 19	0.0002
Glucagon (ng/l)	197 ± 28	203 ± 41	207 ± 25	203 ± 30	168 ± 36	NS
CPIR	2.6 ± 0.3*	3.6 ± 0.5	5.9 ± 0.8*	4.3 ± 0.8	3.5 ± 0.4	0.03
cf normal (%)	74	103	169	123	100	

Data are means ± SE. P values are from one-way ANOVA. *Significantly different from other groups by Student-Newman-Keuls tests.

TABLE 2
Postabsorptive levels of plasma glucose, insulin, C-peptide, glucagon, and C-peptide:insulin concentration (CPIR) ratios at 2 months posttransplant

	REN rats	REN-RMA rats	IH rats	POP rats	Normal rats	*P*
n	11	10	12	8	9	
Glucose (mmol/l)	6.2 ± 0.2	6.0 ± 0.1	6.2 ± 0.1	6.0 ± 0.1	5.7 ± 0.2	NS
Insulin (pmol/l)	151 ± 19*	71 ± 9	74 ± 9	67 ± 6	72 ± 8	0.0001
C-peptide (pmol/l)	262 ± 36	281 ± 38	370 ± 43	254 ± 27	296 ± 39	NS
Glucagon (ng/l)	241 ± 19	210 ± 22	206 ± 25	226 ± 32	172 ± 20	NS
CPIR	1.8 ± 0.2*	4.4 ± 0.7	5.5 ± 0.7	4.1 ± 0.7	4.3 ± 0.5	0.0003
cf normal (%)	42	102	128	95	100	

Data are means ± SE. *P* values are from one-way ANOVA. *Significantly different from other groups by Student-Newman-Keuls tests.

responses and insulin sensitivity in previously diabetic rats with RENs and REN-RMAs (11). In the present study, in addition to OGTTs, glucose turnover was examined in rats with RENs and rats with REN-RMAs during euglycemic hyperinsulinemic clamps, in conditions otherwise identical to those of the previous study. The same comparisons were extended to rats with IHs and to rats with POPs with presumed hepatic portal delivery of secretions.

The postabsorptive state and the responses to an oral glucose challenge in IH and POP rats. All findings were normal in rats with REN-RMAs and POPs. In IH rats, normal plasma glucose and insulin levels in the postabsorptive state and after the glucose challenge suggested normal efficacy of graft-derived insulin. However, abnormally high plasma C-peptide levels in the postabsorptive state, and after the oral glucose challenge, indicated elevated rates of insulin secretion. These results might be related to exposure of the grafts to higher-than-normal concentrations of glucose and gastrointestinal hormones in the local mixture of portal and arterial blood, by comparison with arterial blood. The results of the OGTTs suggested increased MCR of graft-derived insulin (mean 131% of normal rats), apparently due to high intrahepatic extraction in IH rats. The mean MCR of endogenous insulin in the contemporary postabsorptive state was also raised (mean 169% of normal rats).

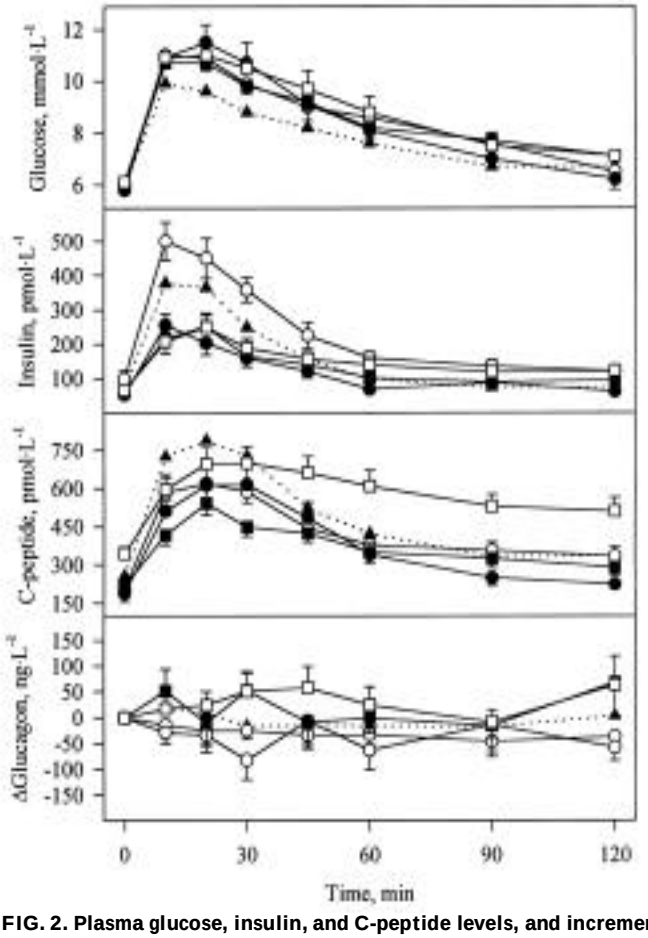

FIG. 2. Plasma glucose, insulin, and C-peptide levels, and increments of immunoreactive glucagon, after a 1.5 g/kg body wt glucose challenge by gavage in 12 REN rats (○), 8 REN-RMA rats (●), 12 IH rats (□), 13 POP rats (■), and 8 normal rats (▲).

MCR and efficacy of exogenous insulin in glucose turnover experiments. Systemic infusion of insulin was used to induce an INS-SS and to estimate the MCR of exogenous insulin. Again, all findings were normal in rats with REN-RMAs and POPs. Reduced MCR of insulin in REN rats, as indicated by the higher INS-SS plasma levels, accorded with our earlier studies in which glucose turnover was not examined (11) and suggested a mean reduction of the MCR to ~50% of normal. This is consistent with the matching of INS-SS insulin levels to those in REN rats attained by doubling the infusion rate of insulin in REN-RMA, IH, and normal rats. The discrepancy between the high MCR of endogenous insulin and the normal MCR of exogenous insulin during the INS-SS in IH rats could be related to different degrees of exposure of the liver to insulin. It appears that the liver in IH rats extracts a large portion of endogenous insulin within the organ when the local concentration in the vicinity of an islet is high, while an abnormality of the MCR of exogenous insulin sufficient to alter whole body clearance of insulin is not present when β-cell secretion is suppressed during systemic administration of exogenous insulin. This acute reversal of the abnormality of MCR of insulin in IH rats contrasts with the persistent reduction of MCR of insulin in the INS-SS in REN rats, which was taken to indicate an adaptation of the clearance mechanism(s) to chronic low hepatic insulin levels (11).

Efficacy of exogenous insulin in stimulation of whole body glucose disappearance. Using the same insulin infusion rate (10 pmol · kg^{-1} · min^{-1}), R_d values were similar among groups despite the fact that REN rats had significantly higher INS-SS insulin levels. This indicated that stimulation of R_d by insulin was impaired in REN rats. The degree of impairment

TABLE 3
AUC (0–120 min) of plasma glucose, insulin, C-peptide, and C-peptide:insulin ratios (CPIR) after a 1.5-g/kg glucose challenge by gavage

	REN rats	REN-RMA rats	IH rats	POP rats	Normal rats	*P*
n	12	8	12	13	8	
Glucose (mol/l · min)	1.0 ± 0.04	1.0 ± 0.05	1.1 ± 0.05	1.0 ± 0.05	0.9 ± 0.03	NS
Insulin (nmol/l · min)	27.3 ± 2.6*	13.8 ± 0.6	18.2 ± 1.8	15.6 ± 1.1	18.8 ± 2.1	0.0002
C-peptide (nmol/l · min)	51.2 ± 3.3	45.7 ± 3.5	70.6 ± 5.8*	44.6 ± 2.9	57.8 ± 5.2	0.0001
CPIR	2.0 ± 0.2*	3.4 ± 0.3	4.2 ± 0.3*	2.9 ± 0.2	3.2 ± 0.3	0.0001
cf normal (%)	63	106	131	91	100	

Data are means ± SE. *P* values are from one-way ANOVA. *Significantly different from other groups by Student-Newman-Keuls tests.

was evident when INS-SS levels were matched to REN rats by doubling the insulin infusion rates in REN-RMA and normal rats, with relative reduction of R_d by more than 40% in REN rats. This abnormality could be due to chronic hyperinsulinemia with systemic delivery of insulin, compared with normal rats. This hypothesis is supported by a study in humans in which insulin was infused systemically to produce hyperinsulinemia for 20 h and insulin sensitivity was dramatically decreased (16). Furthermore, in normal dogs, when insulin was infused portally to produce systemic hyperinsulinemia, maximal glucose utilization was decreased (17). The few data available from diabetic patients after pancreas transplantation with systemic vascular connection, which is associated with hyperinsulinemia, showed that insulin-mediated whole body glucose uptake (18) and muscle GLUT4 content (19) were not normalized. In the present study in IH rats with normal peripheral plasma insulin levels, values for R_d were increased to a normal degree by doubling the infusion rate of exogenous insulin, indicating that there is no significant impairment of the capacity of insulin to stimulate R_d in this model.

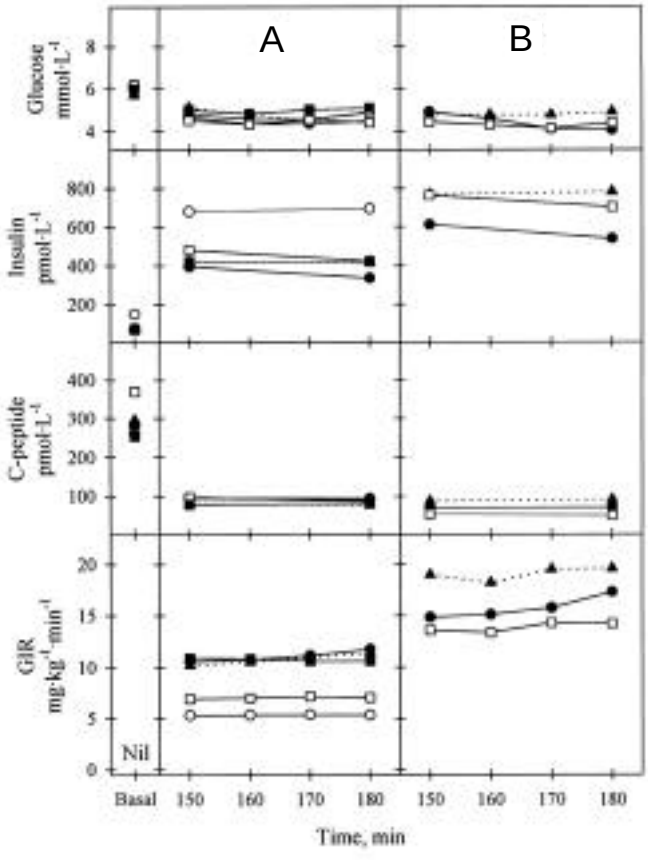

FIG. 3. Plasma glucose, insulin, and C-peptide levels and GIRs in REN rats (○), REN-RMA rats (●), IH rats (□), and POP rats (■) compared with normal rats (▲) in the glucose turnover experiments during the postabsorptive (basal) state and the INS-SS with infusion of 10 pmol · kg^{-1} · min^{-1} insulin (*A*) and of 20 pmol · kg^{-1} · min^{-1} insulin (*B*).

Efficacy of exogenous insulin in inhibition of endogenous glucose production. Using primed constant [3–^{3}H]glucose infusion, we aimed to measure R_d in the INS-SS, which is theoretically equal to the sum of EGP and GIR. Tracer-determined R_d is often less than the GIR during high-dose "cold" glucose infusion. The resulting negative value for EGP is presumably accounted for by dilution of the tracer by unlabeled exogenous glucose (20). Although such dilution may be prevented by empirically determined delivery of additional tracer in dogs (21) and humans (22), that procedure has not been established in rats, and we did not use it in the present study. It has been shown in dogs that the proportionate effect on R_d attributable to such dilution of tracer is relatively small compared with that on EGP (23). Interpretation of the results under these conditions depends on the assumption that EGP is reduced to zero when negative values are obtained (24).

In the present study, false-negative estimates of EGP were obtained in RMA, POP, and normal rats, as well as in IH rats with infusion of insulin at the higher rate. When the negative values for EGP were treated as complete inhibition (EGP = 0) in each animal, the differences described remained statistically significant. We conclude that EGP was virtually completely suppressed during the INS-SS in REN-RMA, POP, and normal rats, and in IH rats with infusion of insulin at the higher rate, and that positive values for EGP in REN rats, and IH rats with infusion of insulin at the lower rate, indicate impaired suppression of EGP by exogenous insulin in these models. Because the liver and kidneys are the organs responsible for EGP in the postabsorptive state, and the major portion of EGP is derived from the liver (25), we infer that hepatic insulin resistance is present in both REN and IH rats, while hepatic responses are normal in REN-RMA and POP rats.

With respect to the finding in REN rats, the notion that hepatic insulin resistance is associated with systemic delivery of insulin is supported by experiments in euglycemic humans with type 1 diabetes after pancreas transplantation with systemic venous drainage (9). Under acute conditions, dependence of suppression of hepatic glucose production on

TABLE 4
Plasma glucose, insulin, C-peptide, and glucagon levels; R_d; GIR; apparent EGP; and the MCR of exogenous insulin during the INS-SS of glucose turnover experiments (mean values of the last 30 min) with an insulin infusion rate of 10 pmol · kg^{-1} · min^{-1} in rat groups

	REN rats	REN-RMA rats	IH rats	POP rats	Normal rats	*P*
n	11	5	6	8	5	
Glucose (mmol/l)	4.6 ± 0.2	4.6 ± 0.4	4.5 ± 0.4	4.9 ± 0.2	4.7 ± 0.2	NS
Insulin (pmol/l)	752 ± 95*	354 ± 68	456 ± 92	393 ± 56	392 ± 71	0.006
C-peptide (pmol/l)	85 ± 6	95 ± 9	92 ± 16	76 ± 13	81 ± 2	NS
Glucagon (ng/l)	242 ± 24	249 ± 24	182 ± 29	217 ± 31	156 ± 45	NS
R_d (mg · kg^{-1} · min^{-1})	7.5 ± 0.6	8.7 ± 1.1	8.2 ± 0.9	9.0 ± 0.4	9.4 ± 0.9	NS
GIR (mg · kg^{-1} · min^{-1})	5.5 ± 1.1*	10.3 ± 0.7	7.0 ± 1.0*	10.5 ± 0.6	10.2 ± 0.8	0.0002
EGP (mg · kg^{-1} · min^{-1})	2.0 ± 0.6*	–1.6 ± 0.4	1.3 ± 0.5*	–1.5 ± 0.3	–0.8 ± 0.4	0.0001
MCR of exogenous insulin (ml · kg^{-1} · min^{-1})	16 ± 2*	32 ± 8	27 ± 5	29 ± 6	30 ± 7	0.02
cf normal (%)	53	107	90	97	100	

Data are means ± SE. *P* values are from one-way ANOVA. *Significantly different from other groups by Student-Newman-Keuls tests.

portal delivery of insulin has not been consistently demonstrable in vivo (26); however, in normal dogs, hepatic glucose output falls more promptly when portal insulin levels are raised in the absence of an increase in arterial insulin than when arterial insulin is raised to the same level in the absence of a rise in portal insulin (27). With chronic systemic delivery of insulin, reduction of hepatic glycogen content in REN rats also suggested that insulin's hepatic action is impaired under these conditions (6). Thus, the present study reinforces the conclusion that chronic systemic delivery of insulin leads to insulin resistance, and it further indicates that insulin's actions to inhibit EGP and to stimulate R_d are both impaired in this condition. The normal effects of insulin on R_d in REN-RMA and POP rats further support the conclusion that their normal insulin sensitivity is dependent on portal delivery of insulin and is not otherwise related to the site of engraftment.

In IH rats, although normal OGT with normal insulin responses suggested normal efficacy of endogenous insulin, hyperinsulinemia in the liver, due to local secretion of insulin by intrahepatic grafts, may have compensated for hepatic insulin resistance. Thus, a lack of high insulin concentrations in the liver during glucose turnover studies with peripheral infusion of insulin may account for the reduction of GIR observed in IH rats. Our results are not necessarily in conflict with a study showing improvement of insulin sensitivity with intrahepatic islet allografts in humans with type 1 diabetes, since the insulin resistance present in this condition before islet transplantation could have confounded interpretation (28). In another study of syngeneic intrahepatic rat islet transplantation, insulin sensitivity was normal (29). However, the supraphysiological doses of insulin used (29 and 72 nmol · kg^{-1} · min^{-1}) could have raised the insulin concentration in the liver to levels similar to those achieved by endogenous insulin secreted by the islets in the liver, again compensating for abnormalities of insulin's action on the liver. Because the stimulation of R_d is normal in IH rats, we conclude that the resistance to exogenous insulin in these animals in our study is largely due to impairment of insulin's effect on EGP, indicating hepatic insulin resistance.

Increased C-peptide under physiological conditions in IH rats. We found elevated plasma C-peptide levels in IH rats in fasting and glucose-fed states, while C-peptide levels approached the assay detection limit during the INS-SS. It may be noted that physiological functions of C-peptide have been proposed (30), and it has been observed that whole body glucose utilization is enhanced by pharmacological dose C-peptide infusion in rats (31). No evidence is available with

TABLE 5
Plasma glucose, insulin, C-peptide, and glucagon levels; R_d; GIR; apparent EGP; and the MCR of exogenous insulin during the INS-SS of glucose turnover experiments (mean values of the last 30 min) in rat groups

	REN rats	REN-RMA rats	IH rats	Normal rats	*P*
n	11	5	6	5	
Insulin infusion rate (pmol · kg^{-1}· min^{-1})	10	20	20	20	
Glucose (mmol/l)	4.6 ± 0.2	4.5 ± 0.2	4.5 ± 0.3	4.7 ± 0.4	NS
Insulin (pmol/l)	752 ± 95	576 ± 24	718 ± 179	785 ± 73	NS
C-peptide (pmol/l)	85 ± 6	70 ± 2	65 ± 17	88 ± 13	NS
Glucagon (ng/l)	242 ± 24	170 ± 18	244 ± 52	195 ± 50	NS
R_d (mg · kg^{-1} · min^{-1})	7.5 ± 0.6*	11.4 ± 0.9	10.5 ± 0.6	13.2 ± 0.7	0.0001
GIR (mg · kg^{-1} · min^{-1})	5.5 ± 1.1*	16.1 ± 0.8	12.6 ± 1.0*	19.0 ± 0.8	0.0001
EGP (mg · kg^{-1} · min^{-1})	2.0 ± 0.6*	–4.7 ± 0.8	–2.1 ± 0.4*	–5.8 ± 0.2	0.0001
MCR of exogenous insulin (ml · kg^{-1} · min^{-1})	16 ± 2*	35 ± 2	28 ± 8	26 ± 2	0.03
cf normal (%)	62	135	108	100	

Data are means ± SE. *P* values are from one-way ANOVA. *Significantly different from other groups by Student-Newman-Keuls tests.

respect to the possible action of C-peptide on hepatic metabolism of glucose. However, if C-peptide serves to maintain or enhance hepatic insulin sensitivity, effects of withdrawal of C-peptide might be more marked in IH animals than in those with lower hepatic C-peptide levels. Therefore, we speculate that C-peptide may play a role in IH rats in maintenance of normal OGT, and that this represents a hepatic action.

Summary. Chronic systemic delivery of insulin in REN rats is associated with hyperinsulinemia, insulin resistance, and impairment of insulin's actions on both hepatic glucose production and extrahepatic glucose utilization. All findings in REN-RMA and POP rats, the parahepatic omental pouch being a potential site for islet transplantation in humans, are normal. Intrahepatic islet transplantation is associated with hypersecretion of insulin, with exaggerated rates of hepatic extraction of graft-derived insulin, and with hepatic insulin resistance in rats. Corresponding data in euglycemic humans with islet grafts are too limited for interpretation. However, the present study shows that intrahepatic islet transplantation does not provide physiological regulation of glucose metabolism in euglycemic glucose-tolerant rats, and it raises the question of whether the liver is an appropriate site for insulin-secreting tissue replacement therapy in type 1 diabetes. The findings also emphasize the importance of hepatic portal delivery of insulin in the development of physiological replacement treatment of diabetes.

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REFERENCES

1. Lickley HLA, Chisholm DJ, Rabinovitch A, Wexler M, Dupré J: Effects of portacaval anastomosis on glucose tolerance in the dog: evidence of an interaction between the gut and the liver in oral glucose disposal. *Metabolism* 24:1157–1168, 1975
2. Williamson MP, Behme MT, Dupre J, Grant DR, Guan J, Zhong R: Rats with portal-caval vein transposition show hyperinsulinemia and insulin resistance. *Metabolism* 45:120–125, 1996
3. Dupré J, Grace DM, McDonald TJ, Inculet R, Leblanc B: Pancreatic endocrine responses to nutrients and bombesin after segmental pancreas autotransplantation in dogs. *Pancreas* 2:99–105, 1987
4. Baumgartner D, Illig R, Sutherland DER: Effects of venous drainage of the vena cava and denervation on endocrine function of pancreatic segments in dogs. *Transplant Proc* 16:769–772, 1984
5. Radziuk J, Barron P, Najm H, Davies J: The effect of systemic venous drainage of the pancreas on insulin sensitivity in dogs. *J Clin Invest* 92:1713–1721, 1993
6. Kruszynska YT, Home PD, Alberti KGMM: Comparison of portal and peripheral insulin delivery on carbohydrate metabolism in streptozotocin-diabetic rats. *Diabetologia* 28:167–171, 1985
7. van Suylichem PTR, Strubble JH, Houwing H, Wolters GHJ, van Schilfgaarde R: Rat islet isograft function: effects of graft volume and transplantation site. *Transplantation* 57:1010–1017, 1994
8. Cuthbertson RA, Mandel TE: A comparison of portal versus systemic venous drainage in murine fetal pancreatic islet transplantation. *Aust J Exp Biol Med Sci* 64:175–184, 1986
9. Rooney DP, Robertson RP: Hepatic insulin resistance after pancreas transplantation in type 1 diabetes. *Diabetes* 45:134–138, 1996
10. Diem P, Abid M, Redmon JB, Sutherland DER, Robertson RP: Systemic venous drainage of pancreatic allografts as independent cause of hyperinsulinemia in type 1 diabetes recipients. *Diabetes* 39:534–540, 1990
11. Guan J, Zucker PF, Behme MT, Zhong R, Atkison P, Dupre J: Insulin resistance prevented by portal delivery of insulin in rats with renal subcapsular islet grafts. *Diabetes* 46:372–378, 1997
12. Kahn BB, Rossetti L, Lodish HF, Charron MJ: Decreased in vivo glucose uptake but normal expression of GLUT1 and GLUT4 in skeletal muscle of diabetic rats. *J Clin Invest* 87:2197–2206, 1991
13. Herbert V, Lau KS, Gottlieb CW, Bleicher SJ: Coated charcoal immunoassay of insulin. *J Clin Endocrinol* 25:1375–1384, 1965
14. Akpan JO, Weide LG, Gingerich RL: A specific and sensitive radioimmunoassay for rat C-peptide. *Int J Pancreatol* 13:87–95, 1993
15. Lewis GF, Steiner G, Polonsky KS, Weller B, Zinman B: A new method for comparing portal and peripheral venous insulin delivery in humans: tolbutamide versus insulin infusion. *J Clin Endocrinol Metab* 78:66–70, 1994
16. Marangou AG, Weber KM, Boston RC, Aitken PM, Heggie JCP, Kirsner RLG, Best JD, Alford FP: Metabolic consequences of prolonged hyperinsulinemia in humans: evidence for induction of insulin insensitivity. *Diabetes* 35:1383–1389, 1986
17. McGuinness OP, Myers SR, Neal D, Cherrington AD: Chronic hyperinsulinemia decreases insulin action but not insulin sensitivity. *Metabolism* 39:931–937, 1990
18. Luzi L, Secchi A, Facchini F, Battezzati A, Staudacher C, Spotti D, Castoldi R, Ferrari G, Di Carlo V, Pozza G: Reduction of insulin resistance by combined kidney-pancreas transplantation in type 1 (insulin-dependent) diabetic patients. *Diabetologia* 33:549–556, 1990
19. Elahi D, McAloon-Dyke M, Clark BA, Kahn BB, Weinreb JE, Minaker KL, Wong GA, Morse LA, Brown RS, Shapiro ME, Gingerich RL, Rosenlof LK, Pruett TL, Andersen DK, Hanks JB: Sequential evaluation of islet cell responses to glucose in the transplanted pancreas in humans. *Am J Surg* 165:15–22, 1993
20. Bergman RN, Steil GM, Bradley DC, Watanabe RM: Modeling of insulin action in vivo. *Annu Rev Physiol* 54:861–883, 1992
21. Fisher SJ, Shi ZQ, Lickley HL, Efendic S, Vranic M, Giacca A: A moderate decline in specific activity does not lead to an underestimation of hepatic glucose production during a glucose clamp. *Metabolism* 45:587–593, 1996
22. Hother-Nielsen O, Henriksen JE, Holst JJ, Beck-Nielson H: Effects of insulin on glucose turnover rates in vivo: iotope dilution versus constant specific activity technique. *Metabolism* 45:82–91, 1996
23. Finegood DT, Bergman RN, Vranic M: Estimation of endogenous glucose production during hyperinsulinemic-euglycemic glucose clamps: comparison of unlabeled and labeled exogenous glucose infusates. *Diabetes* 36:914–924, 1987
24. Radziuk J, Norwich KH, Vranic M: Experimental validation of measurements of glucose turnover in non-steady state. *Am J Physiol* 234:E84–E93, 1978
25. Cersosimo E, Garlick P, Ferretti J: Insulin regulation of renal glucose metabolism in humans (Abstract). *Diabetologia* 40:A24, 1997
26. Rebrin K, Steil GM, Getty L, Bergman RN: Free fatty acid as a link in the regulation of hepatic glucose output by peripheral insulin. *Diabetes* 44: 1038–1045, 1995
27. Sindelar DK, Chu CA, Edgerton DS, Neal DW, Cherrington AD: Basal hepatic glucose production is regulated by the portal vein insulin concentration (Abstract). *Diabetologia* 40:A23, 1997
28. Luzi L, Socci C, Falqui L, Battezzati A, Secchi A, Perseghin G, Maffi P, Bianchi E, di-Carlo V, Pozza G: Successful intrahepatic islet transplantation reverses non-steroid-related insulin resistance in humans. *Transplant Proc* 26:572, 1994
29. Tobin BW, Lewis JT, Tobin BL, Finegood DT: Insulin action in previously diabetic rats receiving graded numbers of islets of Langerhans. *Transplantation* 59:1464–1469, 1995
30. Ido Y, Vindigni A, Chang K, Stramm L, Chance R, Heath WF, DiMarchi RD, Di Cera E, Williamson JR: Prevention of vascular and neural dysfunction in diabetic rats by C-peptide. *Science* 277:563–566, 1997
31. Wu W, Oshida Y, Yang WP, Li L, Ohsawa I, Sato J, Iwao S, Johansson BL, Wahren J, Sato Y: Effect of C-peptide administration on whole body glucose utilization in STZ-induced diabetic rats. *Acta Physiol Scand* 157:253–258, 1996