Comparison of Portal and Peripheral Insulin Delivery on Lipid Metabolism in Streptozocin-diabetic Rats

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SUMMARY

Severely diabetic (150 mg/kg streptozocin, STZ) rats were transplanted with fetal pancreatic islets: (1) under the renal capsule to model peripheral insulin delivery, and (2) into the splenic pulp to model portal delivery. In both groups of transplanted animals, weight gain and blood glucose levels were normal as were peripheral plasma insulin levels. Plasma nonesterified fatty acids, glycerol, acetoacetate, 3-hydroxybutyrate, triglyceride, and cholesterol levels were normal in the two groups of transplanted animals as were VLDL-triglyceride turnover and total ketone body turnover. Adipose tissue lipoprotein lipase activity was also normal in both the fed and fasting states. The findings indicate that consistent normoglycemia and normalization of many aspects of lipid metabolism can be achieved in the rat with peripheral insulin delivery without associated hyperinsulinemia. DIABETES 1985; 34:611–16.

Many metabolic abnormalities are observed in well-controlled diabetic patients during both subcutaneous (s.c.) and intravenous (i.v.) insulin delivery.[1,2] These abnormalities are associated with hyperinsulinemia, and it has been suggested that they may be consequent on the nonphysiologic delivery route.[3] The change in the balance of hepatic to peripheral insulin delivery may account for the suppression of plasma NEFA and glycerol levels[1,2,4] and decreased triglyceride turnover[5] when normoglycemia is achieved and maintained in the short term by peripheral infusion of insulin.

To study longer periods of normoglycemia while delivering insulin peripherally, we have treated STZ-diabetic rats by islet transplantation under the kidney capsule. The metabolism of these animals has been compared both with diabetic rats receiving insulin portally from islets transplanted into the splenic pulp and with normal control animals.

The aim of the present study was to examine whether lipid metabolism was abnormal in normoglycemic rats receiving insulin by the peripheral route in the long term. Studies were chosen to reflect effects on peripheral, hepatic, and overall lipid metabolism.

MATERIALS AND METHODS

Animals. Inbred Wistar albino Boots rats (Nottingham University, Nottingham, United Kingdom) were used both for isolation of pancreatic islets and metabolic studies. Rats were fed on laboratory chow (71% carbohydrate, 22% protein, 7% fat) and housed in a room with equal light and dark cycles commencing at 0700 and 1900 h. Indwelling cannulae (Bard-I-Cath, Bard International, Sunderland, United Kingdom) for metabolic studies were implanted under ether anesthesia in the external jugular and femoral veins 24 h before study. Rats were fed ad libitum unless otherwise stated.

Induction of diabetes and insulin therapy. Diabetes was induced in male rats (110–130 g) by i.v. injection of STZ (0.15 g/kg, in 0.01 mol/L citrate buffer, pH 4.5) under ether anesthesia. Control animals were injected with citrate buffer alone. Diabetes was confirmed by blood glucose estimation (23.7 ± 0.4 mmol/L, mean ± SEM) and weight loss (18.3 ± 0.8 g) at 18 h. Rats were maintained from the first day after STZ by daily s.c. injection of highly purified protamine zinc insulin (PZI, Hypurin, CPI Pharmaceuticals, Wrexham, United Kingdom). After islet transplantation, insulin was continued at 2 U/day for 2 wk. Withdrawal of insulin therapy 4 mo after induction of diabetes in nontransplanted rats led invariably to death from ketoacidosis. Immunoperoxidase staining of the pancreas of STZ-injected islet transplanted rats showed a complete absence of B-cells.[6]

Islet culture and transplantation. Syngeneic pancreatic islets isolated from fetuses at 22 days gestation were cultured as previously described.[7] After 6 days in culture, 5000 islets in a volume of 100 μl of culture medium (RPMI 1640, Flow Laboratories, Irvine, Scotland) were transplanted either un-

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der the kidney capsule to model peripheral insulin delivery, or into the splenic pulp to model portal insulin delivery. Metabolic studies were performed on normoglycemic animals 7–10 wk after transplantation when animals weighed 250–300 g.

Collection of blood samples and analytic techniques. Blood samples were taken from 24-h-fasted, unanesthetised, unrestrained rats with indwelling jugular venous cannulae. Blood glucose was estimated by a glucose-oxidase method (Yellow Springs Glucose Analyser, Clandon Scientific, London, United Kingdom). Plasma insulin was measured by ethanol extraction radioimmunoassay[8] using a rat insulin standard (Novo Research Institute, Bagsvaerd, Denmark). Plasma nonesterified fatty acids (NEFA) were assayed by a radiocobalt method.[9] Blood glycerol was determined on perchloric acid extracts using an automated enzymic fluorimetric method.[10]

Blood samples for lipid assay were collectd in EDTA (1 mg/ml of blood) and the plasma refrigerated immediately. Plasma total cholesterol was determined by an enzymatic colorimetric method (Boehringer Mannheim, Mannheim, FRG) and HDL cholesterol was determined on samples of supernatant after precipitation of apoB-containing lipoproteins with heparin-manganese.[11] Plasma for triglyceride determination was extracted with isopropanol and polar lipid removed with silicic acid. Samples were evaporated to dryness and the triglyceride determined as glyceride glycerol[10] after hydrolysis with alcoholic potassium hydroxide.

For determination of liver triglyceride concentrations, ad libitum-fed rats were killed between 0900 and 1000 h by a blow to the head. Liver was freeze clamped, ground in liquid nitrogen, and stored at −70°C until assayed. A Folch extract was prepared,[12] and liver triglyceride determined as glyceride glycerol as above.

Very-low-density lipoprotein–triglyceride turnover. Rats used for the preparation of labeled VLDL had their drinking water replaced by 100 g/L fructose for 24 h beforehand. Hepatocytes from these animals were isolated by the method of Seglen,[13] except that the liver was perfused in situ and the flow rate maintained at 35 ml/min throughout. Hepatocytes from two livers were incubated (37°C, 95% O_2:5% CO_2), at a final concentration of 6×10^6 cells/ml in Krebs-Hensleit bicarbonate buffer, pH 7.4, containing 20 mmol/L Hepes, 30 g/L bovine serum albumin, 10 mmol/L glucose, 400 mU/L insulin (Human Actrapid, Novo), amino acids (for MEM Eagle [Modified], Flow Laboratories), penicillin 100,000 U/L, streptomycin 0.1 g/L, and oleic acid 1.45 mmol/L with 1.7 mCi/mmol 1-^{14}C-oleic acid (Amersham International, Amersham, United Kingdom). After 4 h, cells were spun down ($800 \times g$, 2 min) and 7.5-ml aliquots of the supernatant were overlayered with 2.5 ml sodium chloride solution (d 1.006) containing 0.1 g/L EDTA. VLDL were isolated by centrifugation at 12°C in a Beckman Ti70 rotor at $105,000 \times g$ for 22 h.

VLDL were removed from the top of the gradient with a Pasteur pipette, concentrated by dry dialysis against polyethylene glycol, and were extensively dialyzed against 0.15 mol/L saline, pH 8.6, before use. ^{14}C-VLDL preparations were characterized by electron microscopy and by SDS-polyacrylamide gel electrophoresis of the delipidated preparation. The distribution of radioactivity in the VLDL lipids was determined by thin-layer chromatography on silica gel plates (Merck, Darmstadt, FRG) using a toluene-ether solvent system. In each experiment, the percentage of radioactivity in the triglyceride moiety was >98%.

VLDL-Triglyceride turnover was determined in 7 kidney capsule islet transplanted, 6 splenic pulp transplanted, and 7 normal control rats. Studies were performed on 24-h-fasted, unanesthetised, unrestrained rats. A basal blood sample was taken at 0900 h for estimation of plasma triglyceride, insulin, and NEFA concentrations. Approximately 2.5 μCi ^{14}C-VLDL in a volume of 300 μl was then given by injection at time 0 min into the femoral cannula and flushed with 300 μl 0.15 mol/L saline. Blood samples were taken from the jugular cannula every 2.5 min until 15 min, then at 20, 25, 30, 45, and 60 min, and replaced with 200 μl of fresh-washed rat erythrocytes in 0.15 mol/L saline.

Plasma was separated immediately and 100 μl added to tubes containing 1.9 ml of isopropanol together with 400 mg silicic acid that had been activated at 120°C for 12 h. Tubes were stoppered, mixed, and left at room temperature for 24 h. Radioactivity of the supernatant after centrifugation was determined in a liquid scintillation counter using an external standard to correct for quenching. Triglyceride as glyceride glycerol was determined on samples of the supernatant as above. Aliquots of ^{14}C-VLDL were treated as for plasma samples for determination of recovery of label (>99%) and for triglyceride content.

The dose administered was <10% of the circulating triglyceride pool. The coefficient of variation of plasma triglyceride concentration during the turnover studies was <5%. Plasma triglyceride specific activity declined monoexponentially ($r = 0.98 \pm 0.01$) until +25 min, by which time specific activity was under 10% of that calculated for time 0 min. Turnover was therefore calculated from the total area under a curve fitted to the observations from +2.5 to +25 min.

Adipose tissue lipoprotein lipase. Lipoprotein lipase activity was measured in the ad libitum-fed and 24-h-fasted states of 10 kidney capsule transplanted, 7 splenic pulp transplanted, and 11 normal control animals. Rats were killed between 0900 and 1000 h by a blow to the head, and epididymal adipose tissue was ground in liquid nitrogen and stored at −70°C. Acetone-ether powders of the ground epididymal fat pads were prepared[14] and stored at −70°C before assay. No loss of activity was observed over a 3-mo storage period under these conditions.

The delipidated powder from one fat pad was homogenized in 5 ml of 5 mmol/L sodium barbital, pH 7.5, containing 20% (vol/vol) glycerol and 0.1% (vol/vol) Triton X-100. The homogenate was centrifuged for 30 min at $60,000 \times g$ at 4°C and the supernatant used for assay by determining the release of nonesterified fatty acids from an artificial triglyceride emulsion (Intralipid, Kabivitrum, Uxbridge, United Kingdom) that had been preincubated at 37°C with horse serum (Oxoid, Basingstoke). The assay was carried out at 30°C in a Tris-albumin buffer, pH 8.1, in a final volume of 1.0 ml.[15] One unit of enzyme activity is defined as the amount of enzyme releasing 1 μmol of NEFA/h in the assay. Enzyme activity was inhibited by >95% in the presence of 1 mol/L sodium chloride and <4% activity was obtained when horse serum was omitted from the system. The intra-assay coefficient of variation was 2.4% and the interassay coefficient of variation was 6.6%.

TABLE 1
Blood glucose and plasma insulin levels in (A) 24-h-fasted and (B) ad libitum-fed normal control and renal capsule or splenic pulp islet transplanted rats

	Normal controls	Renal capsule islet transplanted	Splenic pulp islet transplanted
N	12	10	9
A Glucose (mmol/L)	4.0 ± 0.1	3.8 ± 0.2	3.8 ± 0.1
Insulin (μg/L)	1.45 ± 0.14	1.57 ± 0.13	1.29 ± 0.21
B Glucose (mmol/L)	5.4 ± 0.4	5.4 ± 0.2	5.6 ± 0.4

Mean ± SEM.

Ketone body turnover. Ketone body kinetics were studied in 48-h-fasted rats. Six kidney capsule transplanted, 6 splenic pulp transplanted, and 6 normal control animals were studied. A basal 300-μl blood sample was taken for estimation of plasma NEFA, acetoacetate, and 3-hydroxybutyrate concentrations. At time zero, approximately 20 μCi of 3-^{14}C-3-hydroxybutyrate (Amersham International) in a volume of 150 μl of 0.15 mol/L saline was injected through the injection port of the femoral cannula, and flushed with 300 μl 0.15 mol/L saline. Blood samples were taken at 5, 7.5, 10, 15, 20, 30, 40, 50, and 60 min after administration of tracer, and replaced with 200 μl of fresh-washed rat erythrocytes in 0.15 mol/L saline.

Plasma was separated immediately and 100 μl deproteinized with ice-cold 0.6 mol/L perchloric acid. After centrifugation, an aliquot of the supernatant was taken for 3-hydroxybutyrate estimation by an enzymatic fluorimetric continuous flow assay.[10] The remainder of the acid supernatant was neutralized with 6 mol/L KOH. An aliquot was used for the determination of acetoacetate concentration.[16] Radioactivity in 3-hydroxybutyrate and total ketone bodies was measured by the method of Miles et al.[17] omitting the lyophilization step. Radioactivity was determined in a liquid scintillation counter using an external standard to correct for quenching. Recovery of 3-hydroxybutyrate added to plasma and taken through the whole procedure was 96 ± 1%.

Ketone body turnover was calculated for each experiment assuming steady-state blood ketone body concentrations using the sum of ketone body specific activities.[18,19] The decay in ketone body specific activity closely approximated a double-exponential function (r = 0.98 ± 0.01), and turnover was calculated from the area under the curve.

Statistical analysis. Comparisons between groups were made using Student's unpaired *t*-test. Correlations were sought by the least-squares method. Results are presented as mean ± SEM.

TABLE 2
Twenty-four-hour fasting plasma NEFA, glycerol, triglyceride, and cholesterol concentrations in normal rats and animals with islets transplanted under the renal capsule or into the splenic pulp

	Normal controls	Renal capsule islet transplanted	Splenic pulp islet transplanted
NEFA (mmol/L)	1.00 ± 0.09	0.95 ± 0.08	1.01 ± 0.09
Glycerol (mmol/L)	0.19 ± 0.01	0.19 ± 0.01	0.21 ± 0.02
Total triglyceride (mmol/L)	0.73 ± 0.03	0.73 ± 0.03	0.70 ± 0.03
Total cholesterol (mmol/L)	1.39 ± 0.05	1.41 ± 0.05	1.33 ± 0.04
HDL-Cholesterol (mmol/L)	0.95 ± 0.06	0.97 ± 0.06	0.98 ± 0.07

Mean ± SEM.

TABLE 3
Twenty-four-hour fasting plasma triglyceride concentrations and VLDL-TG turnover in normal control and renal capsule or splenic pulp islet transplanted rats

	N	Triglyceride (mmol/L)	VLDL-TG turnover (μmol/min/kg body wt)
Normal controls	7	0.79 ± 0.07	3.42 ± 0.17
Renal capsule islet transplanted	7	0.79 ± 0.05	3.26 ± 0.09
Splenic pulp islet transplanted	6	0.74 ± 0.08	3.42 ± 0.12

Mean ± SEM.

RESULTS

Blood glucose and plasma insulin levels. Blood glucose and plasma insulin levels in the 24-h-fasted state were similar in the two groups of transplanted animals and the normal controls (Table 1). Blood glucose in ad libitum-fed animals measured on blood taken at 0900 h was also similar in the three groups of animals (Table 1).

Plasma NEFA, glycerol, and lipid concentrations. Table 2 shows the 24-h-fasted plasma NEFA, glycerol, triglyceride, cholesterol, and HDL-cholesterol concentrations in the experimental animals. No significant differences were found between the two groups of experimental animals and the controls.

Liver triglyceride concentrations. Hepatic triglyceride was 9.0 ± 0.5 μmol/g wet wt in the ad libitum-fed state in the normal control rats. Identical concentrations were found in the renal capsule and splenic pulp islet transplanted animals, 9.1 ± 0.8 and 9.2 ± 0.6 μmol/g wet wt, respectively.

TABLE 4
Adipose tissue lipoprotein lipase activity in normal control and renal capsule or splenic pulp islet transplanted rats: (A) ad libitum-fed, (B) 24-h fasted

	Normal controls	Renal capsule transplanted	Splenic pulp transplanted
A Fed (N)	11	10	7
Lipoprotein lipase activity (U/g wet wt)	28.5 ± 1.7	29.6 ± 1.5	27.8 ± 2.6
B 24-h fasted (N)	6	6	7
Lipoprotein lipase activity (U/g wet wt)	10.4 ± 1.0	10.3 ± 0.8	10.2 ± 0.8

Mean ± SEM.

TABLE 5
Forty-eight-hour fasting rat weights, plasma NEFA, ketone body concentrations, and ketone body turnover in normal control (N = 6) and renal capsule (N = 6) or splenic pulp (N = 6) islet transplanted rats

	Rat wt (g)	NEFA (mmol/L)	Acetoacetate (mmol/L)	3-Hydroxy-butyrate (mmol/L)	Total ketone bodies (mmol/L)	3-OHB/ACAC	Ketone body turnover (μmol/min/kg body wt)
Normal controls	259 ± 5	1.14 ± 0.14	0.94 ± 0.03	1.73 ± 0.05	2.67 ± 0.20	1.87 ± 0.11	58.5 ± 8.1
Renal capsule islet transplanted	256 ± 2	1.03 ± 0.08	1.10 ± 0.14	1.74 ± 0.20	2.35 ± 0.31	1.73 ± 0.12	57.3 ± 8.0
Splenic pulp islet transplanted	260 ± 4	1.05 ± 0.10	1.08 ± 0.12	1.83 ± 0.07	2.91 ± 0.28	1.72 ± 0.11	55.6 ± 8.4

Mean ± SEM.

VLDL-Triglyceride turnover. VLDL-Triglyceride turnover (Table 3) in animals receiving insulin peripherally (renal capsule transplanted) was the same as that in splenic pulp islet transplanted and normal control rats.

Adipose tissue lipoprotein lipase activity. Adipose tissue lipoprotein lipase activity in the experimental animals in the 24-h-fasted state is presented in Table 4. No significant difference was found between the two groups of transplanted animals and the controls.

Ketone body turnover. After 48 h of fasting, plasma acetoacetate, 3-hydroxybutyrate, and total ketone body concentrations in rats with islets transplanted under the renal capsule (peripheral insulin delivery) were no different from those of animals with islets transplanted into the splenic pulp (portal delivery) or normal control animals (Table 5). The ratio of 3-hydroxybutyrate to acetoacetate was similar in all three groups, and plasma NEFA concentrations did not differ between the three groups (Table 5). No correlation was found between plasma NEFA concentrations and plasma total ketone body concentrations.

During the turnover studies, acetoacetate specific activity averaged 61 ± 2% of the 3-hydroxybutyrate specific activity, confirming the isotopic disequilibrium noted by others.[18,20,21] The specific radioactivities of acetoacetate decreased with time in parallel with those of 3-hydroxybutyrate, suggesting that maximum specific activities were reached before the first samples were taken at 5 min. Ketone body turnover estimated from the total ketone body specific activity time curve did not differ between the three groups of rats (Table 5). Ketone body turnover rate was linearly related to plasma total ketone body concentrations ($r = 0.69$, $P < 0.001$) (Figure 1).

DISCUSSION

The interpretation of studies of lipid metabolism in insulin-treated diabetic man is complicated by the multiplicity of abnormal influences that inevitably stem from current methods of insulin administration. Thus, not only is a physiologic profile of blood glucose control (and other metabolites) never completely restored in man,[1,2] but in studies on dogs the route of insulin delivery has itself been suggested as a cause of the metabolic disturbance.[4,22] Without feedback control, insulin profiles will inevitably be inappropriate. In well-controlled diabetic patients, fasting serum cholesterol and triglyceride concentrations are generally normal[23-26] but may be suppressed below normal with intensified insulin therapy.[5] In the study of Pietri et al.,[5] decreased plasma lipid levels were associated with decreased VLDL-triglyceride turnover in well-controlled type I diabetic patients treated by continuous subcutaneous insulin infusion. Since neither plasma NEFA nor plasma free insulin concentrations were measured, the underlying mechanisms are uncertain. In other groups of patients treated by continuous subcutaneous insulin infusion, hyperinsulinemia has been demonstrated in association with decreased insulin sensitivity,[27,28] and these abnormalities might be expected to be associated with abnormal regulation of insulin sensitive lipoprotein lipase[29-32] and adipose tissue triglyceride lipase. In addition to decreased NEFA supply, concomitant hepatic hypoinsulinemia may result in increased partitioning of NEFA toward β-oxidation and ketogenesis,[33-35] thus decreasing the output of VLDL.[36]

In our animal model of peripheral insulin delivery, in which blood glucose is normal not only in the fed and fasting states

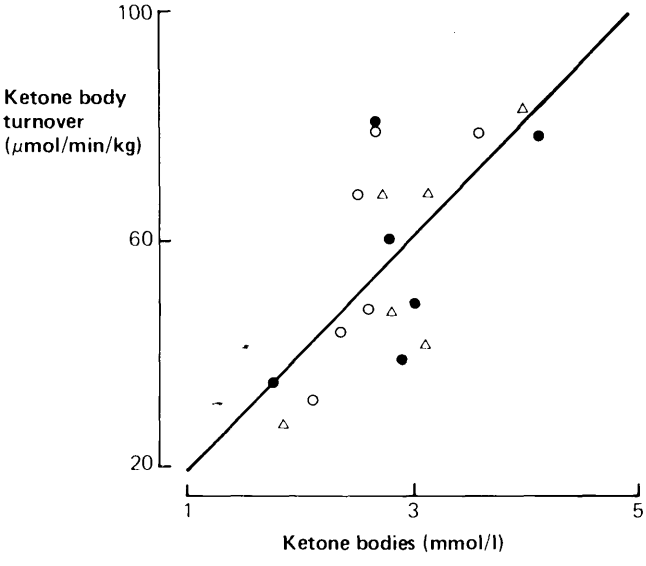

FIGURE 1. Ketone body turnover plotted against plasma ketone body concentration (3-hydroxybutyrate + acetoacetate) for normal control animals (○), and animals given renal capsule (●) or splenic pulp (△) islet transplants. The regression line is fitted to all observations ($y = 20.7x - 1.1$, $r = 0.69$, $P < 0.001$). Individual regression lines did not differ significantly between the three groups of animals.

but also during glucose challenge, the rat is able to maintain normal peripheral insulin concentrations,[37] so that no abnormal influence on peripheral lipid metabolism would be expected. The normal activity of fed and fasted adipose tissue lipoprotein lipase and of plasma NEFA levels are consistent with this.

Normal peripheral insulin concentrations during systemic insulin delivery must imply abnormally low delivery of insulin in the portal vein to the liver. The finding of normal plasma lipid concentrations and of normal VLDL-triglyceride turnover suggests, however, that hepatic production of VLDL is not decreased, while confirming normal peripheral utilization.

Ketone body production will be influenced by insulin-sensitive events both in the periphery[38] and at the liver.[33, 35] Again, however, normal peripheral insulin concentrations will result in normal peripheral lipolysis and hence plasma NEFA concentrations, as demonstrated, so that substrate supply for ketogenesis would not be disturbed in our peripherally treated animals. As with VLDL production, it appears that the liver was capable of maintaining normal rates of ketogenesis despite presumed portal venous hypoinsulinemia.

The results suggest that in the rat, exposure of the liver to insulin concentrations similar to those seen in the systemic circulation is adequate for normal regulation of enzymes of lipogenesis and ketogenesis, and that portal vein insulin concentrations in excess of these are not necessary for normal hepatic NEFA metabolism. Indeed, normal hepatic NEFA supply and, by implication, normal peripheral insulin concentrations may be of primary importance in achieving normal lipid metabolism in the fasted state.

If these results in rats can be extrapolated to man, then it could be suggested that appropriately regulated peripheral insulin delivery in diabetic patients would not itself result in abnormalities of lipid metabolism.

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