Originals

Islet transplantation reverses carcass protein loss in diabetic rats without inducing disproportionate fat accumulation

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Summary The Diabetes Control and Complications Trial demonstrated that intensive insulin therapy (IIT) improves many secondary complications of insulin-dependent diabetes mellitus. However, weight gain in IIT is associated with increased body fat, and no improvement in lean body mass. In the present study we investigated the effects of experimental diabetes on changes in body composition and probed the benefit of glycaemic control achieved through islet transplantation. Male Wistar Furth rats (weight 273 ± 9 g) made diabetic for 2 weeks with streptozotocin (55 mg/kg) were infused intraportally with 3265 ± 692 (150 μ m islet equivalent units) syngeneic islets of Langerhans. Body composition was evaluated by proximate analysis in carcasses of transplant rats (Trans), and also in rats made diabetic for 2 or 7 weeks (Db-2, Db-7) and in 2- and 7-week sham controls (Sham-2, Sham-7). Fed plasma glucose levels were 7.3 ± 1.1 , 28.2 ± 2.4 , 26.8 ± 3.9 , 7.5 ± 1.0 and 7.0 ± 0.1 mm/l, respectively, and neither glucose tolerance nor fasting plasma insulin differed between control vs transplant rats ($p > 0.05$). Two weeks of diabetes resulted in a body weight 82 % of that of con-

trols (240 ± 5 vs 292 ± 8 g, $p < 0.05$) and 5 subsequent weeks of diabetes further suppressed growth by an additional 12 % ($p < 0.05$). Five weeks following islet transplantation, islet-transplant rats had regained lost weight and were not significantly different from control animals (274 ± 19 vs 291 ± 21 g, $p > 0.05$). Body composition analysis indicated that protein mass was virtually completely regained by 5 weeks post-transplantation (Db-2 = 39.6 ± 0.9 , Trans = 46.8 ± 4.7 , Sham-7 = 49.6 ± 2.9 g; $p > 0.05$: Trans vs Sham-7), and fat mass of transplant rats was 75 % that of control values ($p < 0.05$). These data illustrate that the near physiologic glycaemic control achieved through pancreatic islet transplantation is associated with normalization of lean body mass accretion in experimental diabetes and does not induce a disproportionate accumulation of body fat. [Diabetologia (1995) 38: 881–888]

Key words Diabetes mellitus, islet transplantation, blood glucose, insulin secretion, body composition, body protein, body fat, nutrition.

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Abbreviations: IIT, Intensive insulin therapy; DCCT, Diabetes Control and Complications Trial; Db, diabetic; Trans, transplant; IDDM, insulin-dependent diabetes mellitus; HBSS, Hanks Balanced Salt Solution; JV, jugular vein; FA, femoral artery; AIRg, acute insulin response to glucose; K_g , glucose tolerance; RIA, radioimmunoassay; ANOVA, analysis of variance; TBBM, total body bone mineral.

The Diabetes Control and Complications Trial (DCCT) demonstrated a dramatic reduction in selected renal, retinal, and neurological complications of diabetes mellitus [1, 2]. However, in these trials intensive insulin therapy (IIT) was associated with two detrimental side effects: increased hypoglycaemic episodes and weight gain [3, 4]. The weight gain associated with IIT has recently been elucidated, and involves primarily a disproportionate gain in body fat. Patients receiving IIT demonstrated decreased glycosuria, decreased protein oxidation and fat oxidation and a concomitant increase in carbohydrate oxida-

Fig. 1. Plasma glucose level during 2 weeks of diabetes and following pancreatic islet transplantation. Animals made diabetic for 7 weeks (○) are shown with previously diabetic rats that subsequently received transplants of islets of Langerhans (●) and are contrasted to 2-week (▽) or 7-week sham controls (▼). Streptozotocin was administered i. v. (55 mg/kg) at week 0, and animals developed diabetes for a 2-week period. Sham-treated animals received acetate buffer only. In week 2, six diabetic rats were transplanted intraportally with 3265 ± 692 syngeneic islets of Langerhans resulting in normalization of blood glucose level within 1 week. All other animals received a sham transplant of islet carrier vehicle (M-199) only. Values are means $\pm$ SD, $n = 6$ per group

tion [5]. Thus, the net caloric balance favoured an energetic gain in the form of excess body fat.

The rationale for IIT is predicated on the hypothesis that more precise control of plasma glucose will be necessary to ameliorate or control long-term complications of diabetes. However, the demonstration of increased hypoglycaemic episodes and disproportionate weight gain as body fat, underscores the ultimate need for a more physiologic control of glucose than is presently obtained with IIT. Thus, investigations of alternate insulin delivery strategies such as islet transplantation, remain viable paradigms to experimentally delineate the advantages of more physiologic insulin delivery systems upon the secondary complications of insulin-dependent diabetes mellitus (IDDM).

That islet transplantation can ameliorate or reverse selected complications of IDDM in experimental diabetes is well-established [6]. The benefits of islet transplantation include, but are not restricted to, improved renal function [7], amelioration of retinal capillary leakage [8], correction of autonomic nervous system dysfunction [9], and reversal of cardiac lesions [10]. Episodes of idiopathic hypoglycaemia due to erratic or uncontrolled insulin secretion have not been reported in the literature. In addition, near-physiologic control of plasma glucose has been demonstrated even in stress paradigms such as exercise [11]. Taken together, these data illustrate that islet transplantation has the potential to result in independence from exogenous insulin, normalize glycaemic control even in individuals with active lifestyles, and ameliorate many of the secondary complications of IDDM.

One of the consistently demonstrated benefits of islet transplantation is that the weight loss associated with experimental diabetes is rapidly regained [12–

14]. Even diabetic animals transplanted with a suboptimal islet mass will begin to regain body weight, although fed-state normoglycaemia has not been achieved [15]. While reversal of diabetes-related growth deficits have been described by a number of laboratories, the body composition of weight gain following pancreatic islet transplantation has not been elucidated.

The purpose of the present investigations was to characterize the weight gain associated with reversal of experimental diabetes following intraportal islet transplantation. We hypothesized that the near-normal physiologic control of plasma glucose achieved through pancreatic islet transplantation would be associated with a proportionate gain in body fat and lean body mass. The present experiments demonstrate the effect of glycaemic control achieved through pancreatic islet transplantation, and its subsequent effect upon protein, fat, and mineral content of the carcasses of male rats in a model of streptozotocin diabetes.

Materials and methods

Animals. Male Wistar Furth rats (Harlan Sprague-Dawley, Indianapolis, Ind., USA) were obtained at 9–10 weeks of age, approximately 220 g body weight, and housed individually in shoebox cages with cellulose bedding material. Animals were maintained on a 12-h light/dark cycle (lights on 07.00 hours) and had access to food (Harlan Teklad, # 7002, 6 % Mouse/Rat Diet) and water ad libitum. All procedures were carried out in accordance with the guidelines of the National Institutes of Health and the Institutional Animal Care and Use Committee of Mercer University School of Medicine.

Monitored parameters. Plasma glucose was monitored weekly in non-fasted animals (at 12.00–14.00 hours). Tail vein blood samples were collected into 150 μ l heparinized Eppendorf

tubes, placed on ice, and centrifuged within 15 min to separate plasma. Samples were stored at -20°C for future glucose analysis.

Diabetes induction. Following 1–2 weeks of acclimation, animals were randomly assigned to treatment groups when body weight was approximately 265–280 g. Animals designated for diabetes or islet transplantation were anaesthetized with 60 mg/kg sodium pentobarbital (Somnotol) and injected i.v. (penile vein) with 55 mg/kg streptozotocin (Sigma, St. Louis, Mo., USA) dissolved in acetate buffer. Control animals were also anaesthetized, but were injected with acetate buffer only. Diabetes was allowed to develop for a 2- or 7-week period (Fig. 1).

Donor islet isolation. Donor male Wistar Furth rats (approximately 250 g) were fasted overnight and prepared for pancreatectomy following 60 mg/kg i.p. sodium pentobarbital injection. Islets were isolated using the methods of Ballinger and Lacy [16] with modifications as described previously [15]. Briefly, the pancreas distended with Hanks balanced salt solution (HBSS, Sigma), was excised, minced, and the islets were digested from connective tissue with type V collagenase (Sigma, lot number 071H6840). We washed the islets in cold HBSS, purified them on a ficoll gradient, and placed them into Medium-199 (Sigma). Purified islets were visualized under $250\times$ magnification with a green illuminated background and white fibre optic side illumination. Using a glass micro-pipette we hand-picked islets to obtain grafts containing approximately 3000 islets. The average islet diameter of each graft was estimated by sampling a portion of the graft suspended in Medium-199. We quantified islet diameter against an optical graticule; islet number per graft was calculated as 3265 ± 692 ($150\text{ }\mu\text{m}$ islet equivalent units) according to standards established by Ricordi et al. [17].

Islet transplantation. After 2 weeks of diabetes (Db), six animals were prepared for islet transplantation following an overnight fast. Donor islets were infused into the portal vein in a carrier of Medium-199 while the animal was under sodium pentobarbital anaesthesia (20 mg/kg). Residual islets in the infusion apparatus were quantified to verify a successful graft infusion. Sham control rats ($n = 6$) as well as those animals destined for 2 ($n = 6$) or 7 weeks of diabetes ($n = 6$) were infused intraportally with Medium-199 only.

Preparation for in vivo determinations. Three weeks following islet transplantation we implanted chronic indwelling catheters into the right external jugular vein (JV) and the left femoral artery (FA). Catheters were tunnelled subcutaneously and exteriorized dorsally on the head, housed in a protective plastic cap as previously described [18]. The catheters were filled with heparinized saline (10 U/ml), and were sealed. To limit experimental stress upon short-term animals, those rats destined for only 2 weeks of diabetes were sham operated and the JV and FA were ligated.

One week following catheter surgery, animals were fasted overnight with food removed at 22.00 hours (3 h into dark cycle) to allow for normal cyclic feeding and preclude a state of semi-starvation during experimental protocols. On the morning of in vivo determinations, animals were brought into the laboratory and placed in a plexiglass experiment box ($25 \times 25 \times 11\text{ cm}$) with fresh bedding and a continuous air supply ($3\text{--}4\text{ l/min}$). Catheters were flushed with heparinized saline and connected to extensions of PE-50 tubing. Animals were acclimated to the laboratory environment for 30 min prior to experiments.

Sample handling. Blood samples for plasma glucose and insulin (100 μl) were placed into tubes containing sodium heparin (10 U) and placed immediately on ice. The plasma was separated by centrifugation ($12,700 \times g$, 5 min, Damon/IEC Microfuge, Neddham Hts., Mass. USA) and was subsequently stored at -20°C until the time of analysis.

Insulin secretion and glucose tolerance. One week following catheter surgery, insulin secretion, glucose tolerance, and the acute insulin response to glucose (AIRg), were measured in fasted animals (10 h) following an i.v. bolus of glucose (0.3 g/kg, 10% solution). Blood samples were obtained by arterial catheter for plasma glucose at -10 , -5 , 1, 2, 3.5, 5, 6.5, 8, 10, and 15 min and for plasma insulin at -10 , -5 , 1, 2, 3.5, and 6.5 min post-glucose injection (0 min). Blood samples for plasma glucose and insulin analysis (200 μl) were placed into tubes containing sodium heparin (10 U) and placed on ice. Plasma was separated by centrifugation ($12,700 \times g$, 5 min) and was stored at -20°C until analysis.

Sample analysis. Plasma glucose from monitored samples and glucose tolerance tests was analysed by glucose oxidase method using a Beckman Glucose Autoanalyzer II (Brea, Calif., USA). Plasma insulin was determined in samples from glucose tolerance tests by double antibody competitive binding radioimmunoassay (RIA) (Linco, St. Louis, Mo., USA) using rat antibody and rat insulin standards. The within-run coefficient of variation for the insulin assay was 5.6% for six assays.

Body composition measures. One week following intravenous glucose tolerance tests animals were exsanguinated and prepared for measures of body composition while under sodium pentobarbital anaesthesia. The heart and lungs were removed, the peritoneal cavity was excised of the gastrointestinal tract, liver and kidneys, leaving the epididymal and retroperitoneal fat pads intact. The paws and tail were clipped from the animals as previously described to facilitate a more homogenous grinding and sampling of carcasses [19]. The animals were weighed, then frozen at -70°C until the time of analysis.

Three aliquots of finely ground and homogenized samples were used for proximate chemical analysis [20]. Dry matter was determined following lyophilization and oven drying at 105°C . Protein was determined by the micro-Kjeldahl method, and total fat by the Foss-let procedure [21]. Mineral content was determined by ashing the samples in a muffle furnace (470°C) for 18 h.

Statistical analysis

We calculated the acute insulin response to glucose (AIRg) as the peak insulin concentration observed at 1, 2, or 3.5 min following glucose injection, minus the average pre-stimulus insulin concentration. Glucose tolerance (K_g) was determined as the slope of the log of the glucose concentration vs time following i.v. glucose injection, using the six blood glucose samples obtained during the 2–10 min post-injection. Differences in the dependent variable responses between groups were assessed using a one-way analysis of variance (ANOVA) (NCSS, Version 5.03, Kaysville, Utah, USA), with a post-hoc Duncan's test. All comparisons were considered statistically significant at $p < 0.05$.

Fig. 2. The change in body weight prior to treatment, during 2 weeks of diabetes and following pancreatic islet transplantation or sham treatment. Animals diabetic for 2 ($\diamond$) or 7 weeks ($\circ$) are shown with previously diabetic rats that subsequently received transplants of islets of Langerhans ($\bullet$) and are contrasted to 2-week (∇) or 7-week sham controls ($\blacktriangledown$). The loss of body weight after streptozotocin administration was equivalent in all three diabetic groups ($p > 0.05$). Following islet transplantation, previously diabetic rats rapidly regained lost body weight, and by 5 weeks were not significantly different from sham-treated controls ($p > 0.05$). Values are mean $\pm$ SD, $n = 6$ per group

Table 1. Effect of diabetes and islet transplantation on fasting plasma glucose, basal insulin concentration, insulin secretion, and glucose tolerance in animals treated for 7 weeks

	Fasting plasma glucose (mmol/l)	Fasting plasma insulin (pmol/l)	AIRg (pmol/l)	Kg (%)
Db-7	16.8 ± 6.8^a	58 ± 39^a	18 ± 30^a	0.7 ± 0.4^a
Transplant	7.0 ± 0.6^b	267 ± 59^b	1376 ± 294^{ab}	2.3 ± 0.3^b
Sham-7	6.6 ± 0.5^b	266 ± 107^b	2065 ± 634^b	2.5 ± 0.5^b
ANOVA ($p <$)	0.0006	0.0002	0.0001	0.0001

Values are mean $\pm$ SD, $n = 6$ per group. Significant differences between treatments are noted by superscripts as: ^a vs Sham-7; or ^b vs Db-7, by post-hoc Duncan's test ($p < 0.05$)

Results

Samples for determination of plasma glucose were collected by tail vein sampling on a weekly basis in non-fasted animals (Fig. 1). Plasma glucose concentration increased to greater than 26 mmol/l in diabetic rats, yet was not significantly different between long-term diabetic animals and those that subsequently received transplants of islets of Langerhans ($p > 0.05$). Following islet transplantation the plasma glucose concentration returned to a value not significantly different from that of controls within 1 week following transplant or sham surgery ($p > 0.05$).

There were no significant differences in body weight between animals destined for diabetes, islet transplants or sham control designation prior to experimental treatment (Fig. 2). There was a significant loss of weight in streptozotocin-treated animals (approximately 34 g) through the third (vs first) week ($p < 0.05$); however, sham-treated controls gained on average approximately 20 g ($p < 0.05$). At this point, animals were sham treated or transplanted with freshly isolated islets of Langerhans from syngeneic pancreas donors. Venous and arterial catheters were placed in week 5. In week 6 an intravenous glucose

tolerance test was performed, and in week 7 the animals were killed for body composition analysis. Following catheter surgery there was an equivalent 5% loss of body weight during the following 2 weeks in both transplant and control animals ($p < 0.05$), while diabetic rats declined by 13% ($p < 0.05$, by one-tail paired t -test).

An intravenous glucose tolerance test was performed in week 7, at 4 weeks post-islet transplantation (Table 1). There were no significant differences in the fed (Fig. 1) or fasting plasma glucose levels of sham-treated or islet-transplant animals; however, both the fed and the fasting plasma glucose concentrations were significantly elevated in diabetic animals ($p < 0.05$). The acute insulin response to glucose (AIRg) was calculated as the incremental increase in plasma glucose above baseline during the 3.5 min following i.v. glucose injection (Table 1). However, with the exception of one diabetic animal, all rats exhibited a peak insulin response during the first 2 min post-injection. The AIRg of transplant animals was 66% of control values; however, glucose tolerance did not differ between transplant and control rats. When contrasted to sham controls, the AIRg and Kg of diabetic animals were reduced 99%

Table 2. Body composition in animals made diabetic for 2 or 7 weeks contrasted to animals receiving an intraportal islet transplant of approximately 3000 islets vs sham controls

	Carcass weight (g)	Carcass water (%)	Ash (% DM)	Protein (% DM)	Fat (% DM)
Db-2	152 ± 5 ^a	67.7 ± 0.2 ^a	15.2 ± 1.9	80.7 ± 2.9 ^a	7.8 ± 0.6 ^a
Sham-2	219 ± 7 ^b	65.7 ± 0.7 ^b	9.6 ± 0.7 ^{ab}	65.7 ± 1.9 ^b	22.9 ± 1.6 ^b
Db-7	114 ± 15 ^{ab}	68.0 ± 1.3 ^a	21.5 ± 2.6 ^{ab}	78.0 ± 4.2 ^a	5.1 ± 0.8 ^{ab}
Transplant	206 ± 15 ^b	66.4 ± 0.8 ^{ab}	12.3 ± 0.9 ^b	67.7 ± 3.7 ^b	20.0 ± 1.9 ^{ab}
Sham-7	220 ± 18 ^b	65.1 ± 1.4 ^b	13.0 ± 2.5	64.7 ± 4.2 ^b	23.3 ± 4.2 ^b
ANOVA (<i>p</i> <)	0.0001	0.0001	0.0001	0.0001	0.0001

DM, Dry matter of the animals' carcass, determined by lyophilization and desiccation. Values are mean ± SD, *n* = 6 per group. Significant differences between treatments are noted by superscripts as: ^a vs Sham-7; or ^b vs Db-2 by post-hoc Duncan's test (*p* < 0.05)

Fig. 3. The amount of protein or fat in the carcasses of animals diabetic for 2 or 7 weeks as contrasted to islet-transplant rats and 2- or 7-week sham controls. Animals treated for 2 weeks (■) and 7 weeks (□) of treatment. Treatment means are denoted as significantly different (*p* < 0.05, by post-hoc Duncan's test) when contrasted to Db-2 (*) or Sham-7 controls (#). Carcass protein content was significantly reduced vs controls at 2 and 7 weeks of diabetes (*p* < 0.05). However, the protein content of transplant animals was not different from controls when contrasted at 5 weeks post-islet transplantation (*p* > 0.05). Similarly, fat content in the carcasses of diabetic rats was lesser than controls both at 2 and 7 weeks of diabetes and was virtually completely regained at 5 weeks post-islet transplantation (*p* < 0.05). Values are mean ± SD, *n* = 6 per group

Table 3. Measures of carcass ash content in animals diabetic for 2 or 7 weeks contrasted to animals receiving an intraportal islet transplant of approximately 3000 islets vs sham controls

	Ash (g)	Ash:BW (g/g × 10 ²)	Ash:protein (g/g × 10 ²)
Db-2	7.4 ± 0.8 ^a	3.2 ± 0.3	19.2 ± 2.3
Sham-2	7.3 ± 0.6 ^a	2.5 ± 0.2 ^{ab}	14.7 ± 1.3 ^{ab}
Db-7	7.8 ± 1.1 ^a	4.0 ± 0.7 ^b	28.0 ± 2.9 ^{ab}
Transplant	8.5 ± 0.5	3.1 ± 0.3	18.2 ± 2.1
Sham-7	10.0 ± 2.6 ^b	3.4 ± 0.7	20.1 ± 4.2
ANOVA (<i>p</i> <)	0.0096	0.0008	0.0001

Values are mean ± SD, *n* = 6 per group. Significant differences between treatments are noted by superscripts as: ^a vs Sham-7; or ^b vs Db-2 by post-hoc Duncan's test (*p* < 0.05)

and 72 %, respectively, at 7 weeks of diabetes (*p* < 0.05).

One week following the glucose tolerance test, animals were killed and body composition was subsequently determined by proximate analysis in eviscerated animal carcasses (Table 2). During the 5 additional weeks of diabetes, Db-7 animals engendered a 25 % reduction in carcass weight, while transplant an-

imals increased carcass weight by 26 % (*p* < 0.05, vs Db-2). The greatest percentage of carcass water was demonstrated in Db-7 rats who had 68.0 % carcass water content vs sham controls who had 65.1 % water (*p* < 0.05). In diabetic animals, the percentage ash content was greater than controls, with the greatest percentage seen in Db-7 rats (*p* < 0.05). The percentage carcass protein content was also greatest in diabetic animals; however, there were no significant differences between the Db-2 and Db-7 rats (*p* > 0.05). As would be expected from the hypercatabolic diabetic state, the percentage of carcass fat was dramatically lower in diabetic rats when compared to controls or islet-transplant animals, and was also lower in Db-7 than Db-2 animals (*p* < 0.05).

The contribution of carcass protein and fat to the increased growth of the islet transplanted rat is illustrated in Figure 3. Within the 5 additional weeks of diabetes, the Db-7 animals exhibited a protein mass 28 % less than that of Db-2 animals (*p* < 0.05). Five weeks following islet transplantation these previously diabetic rats demonstrated a 15 % greater protein mass than Db-2 rats and a carcass protein content that was 175 % of Db-7 values (*p* < 0.05). Car-

cass fat mass was dramatically less in diabetic animals and was approximately 10 g greater in islet-transplanted animals compared to Db-2 animals ($p < 0.05$). At 5 weeks post-islet transplantation, there were no significant differences in the carcass protein content of islet-transplanted rats when compared to their sham-treated counterparts ($p > 0.05$).

The ash content of the experimental animals is depicted in Table 3. Two weeks of diabetes was not sufficient time to engender a significant difference in this indicator of the total body bone mineral (TBBM) content [22] of diabetic animals. However, following 7 weeks of diabetes, the ash content of Db-7 carcasses was 22 % less than that of Sham-7 control animals ($p < 0.05$). The ash content of transplant animals was intermediate, not different from diabetic or control animals ($p > 0.05$). A contrast of the ash to body weight or protein weight indicates that 2 weeks of diabetes results in a relative gain in the proportion of TBBM content; however, only the ash:protein ratio could be concluded as significantly greater than control at 7 weeks of diabetes ($p < 0.05$).

Discussion

In the present studies we evaluated changes in body weight during 2 or 7 weeks of diabetes and contrasted carcass composition to animals receiving an intraportal islet transplant of approximately 3000 syngeneic islets of Langerhans. The present studies illustrated that the weight loss associated with experimental diabetes is reversible through islet transplantation. This is an observation which has been consistently demonstrated by a number of laboratories [12–15] since landmark investigations in islet transplantation were performed by Ballinger and Lacy [16]. In the present studies, however, we additionally demonstrated that the regain of body weight following islet transplantation is associated with an increase in both body protein and body fat content. Thus, these are the first investigations to demonstrate that glycaemic control resulting from pancreatic islet transplantation is associated with a relatively proportionate regain in the constituents of body weight lost during 2 weeks of experimental diabetes.

That islet transplantation is not associated with a disproportionate fat accumulation at the expense of protein accretion differs from results demonstrated in human subjects on IIT. In the DCCT, the two primary detrimental consequences of IIT were ascribed to hypoglycaemia and weight gain. Subsequent studies by Carlson and Campbell [5] have elegantly delineated the energetic components of weight gain in patients on IIT. The disproportionate fat accretion associated with this therapy can be ascribed to a shift in energy balance; 70 % of this positive energy balance

is explained by a reduction in glycosuria, and the remainder is attributed to a reduction in the resting metabolic rate. In addition, IIT was associated with a decreased protein and fat oxidation and an increase in carbohydrate oxidation. Since the net caloric balance favoured energy gain, when coupled with a diminished fat oxidation, the decreased resting metabolic rate promoted a disproportionate accumulation of body fat.

The energetic components of weight gain following islet transplantation have not been investigated by any research group. However, data from previous investigations suggest that a portion of the benefit associated with islet transplantation may be explained by a reduction in caloric intake concomitant with improved glycaemic normalization. Our previous studies illustrated that within 3 weeks following transplantation of approximately 3000 islets, previously diabetic rats completely normalized food intake when compared to sham-treated controls [23]. Thus, even though these animals demonstrated an AIRg 60 % of that of controls [15, 23], a magnitude similar to the present study, food intake of islet-transplanted rats was not different from that of controls ($p > 0.05$; 19 ± 1 vs 18 ± 1 g/day). One notable recommendation from the studies of Carlson and Campbell [5] was that IIT patients should be instructed to reduce caloric intake in an effort to offset weight gain. Given the historically demonstrated failure of current weight reduction/maintenance strategies [24], it seems reasonable to assume that weight gain will not be easily managed long-term, in patients on IIT. Thus, it is encouraging that islet transplantation offers an additional advantage, since intraportal islet transplants in previously diabetic rats are associated with a reduction in food intake to a level not different from that of controls within 3 weeks following glycaemic normalization.

The anabolic effects of insulin per se upon protein metabolism are well-documented; thus, the improved protein mass in the animals of the present studies was not unanticipated. As early as 1933, Walsh and colleagues illustrated that protein wasting in IDDM could be eliminated by administration of insulin [25]. Later studies suggested that the degree of metabolic control observed in uncontrolled diabetes may be a determinant of the degree of protein wasting [26]. However, a single mechanism of action which describes the exact nature of the effect of insulin on proteolysis or proteogenesis, remains to be elucidated. The decrease in skeletal muscle protein synthesis observed in diabetes may be related to a decreased number and translational efficiency of ribosomes [27, 28] and may additionally involve alterations in peptide chain elongation/termination [29]. Some manifestations may be organ related, for in the liver, specific secretory proteins such as albumin seem to be particularly effected by insulin defi-

ciency. The data of the present studies illustrate that streptozotocin diabetes of 2 weeks' duration resulted in a carcass protein mass which was 80 % of that of controls at 2 weeks and 57 % of that of controls at 7 weeks. These data demonstrate time-dependent effects which promote further loss of carcass protein content in longer duration diabetes. Islet transplantation, however, resulted in a regain of lost protein to a level which was 94 % of and not statistically different from that of controls ($p > 0.05$). Thus, not only was islet transplantation successful in arresting further protein loss, it was also associated with a regain in carcass protein to normal level as contrasted to age-matched control animals. The present model of protein loss and regain may serve as a useful paradigm to elucidate the catabolic and anabolic effects of insulin, while discriminating effects of mode of insulin administration. In the present paradigm, normalizing the basal insulin concentration may be important to up-regulating protein synthesis, since transplant animals demonstrated equivalent fasting plasma insulin concentrations, yet exhibited an AIRg which was only 66 % of that of control values. Future studies will elucidate the mechanisms of protein gain in islet-transplanted animals, to determine the independent effects of additional factors such as energetic or dietary influences and interactions of islet mass upon carcass protein accretion in experimental diabetes.

In addition to demonstrating alterations in protein and fat, the present animals had a lesser TBBM content following 7 weeks of diabetes than did sham-treated controls. These data suggest that the TBBM content of the diabetic animals was attenuated either by hyperglycaemia or insulinopenia. That IDDM is associated with altered bone mineralization has been documented both in humans [30, 31] and in laboratory animals [32]. Bouillon [33] hypothesized that the decreased bone mineralization of IDDM is the result of a negative calcium balance induced by a decrease in vitamin-D receptors, intestinal calcium absorption, renal 1- α hydroxylase activity, osteoblast number, and serum osteocalcin. The resultant osteopenia leads to bone fragility in IDDM. Insulinopenia per se is proposed to be the primary defect, and a portion of the aetiology of diabetic osteopenia may be secondarily mediated via decreased growth hormone and insulin-like growth Factor-1 (IGF-1), with a concomitant androgen deficiency or resistance. The present data demonstrated that during 7 weeks of diabetes streptozotocin-treated animals failed to achieve the TBBM content of age-matched control animals. Yet, there was no net decrease in the amount of mineral content when contrasted with Sham-2 rats. Thus, these data are consistent with the observation in humans of a deficit in bone mineralization at the time of clinical onset of IDDM [30]. The present data support the hypothesis that an early ef-

fect of diabetes is to inhibit bone accretion during growth periods. Unfortunately, 5 weeks of glycaemic control achieved through pancreatic islet transplantation did not significantly affect the TBBM content; however, the ash:body weight and the ash:protein ratio was normalized by islet transplantation. Whether allowing for a longer duration of growth post-islet transplantation or whether transplanting a greater mass of islets would favour a more complete normalization of TBBM content in previously diabetic rats, remains to be determined.

In summary, 2 weeks of streptozotocin diabetes was associated with a reduction in body weight which was corrected following intraportal pancreatic islet transplantation. The gain in body weight during the 5 weeks post-transplant surgery was explained by a gain in both carcass protein and fat content. Additionally, 7 weeks of diabetes was associated with a 22 % lesser TBBM content, which was marginally, but not statistically improved with islet transplantation. These studies illustrate that the weight gain following pancreatic islet transplantation does not involve a disproportionate fat accretion, but is associated with a proportional gain in both body protein and body fat. Thus, islet transplantation in rats appears to affect body composition in a manner different from that observed in diabetic patients receiving intensive insulin therapy.

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References

1. The DCCT Research Group (1993) The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus: the Diabetes Control and Complications Trial. *N Engl J Med* 329: 977-986
2. Santiago JV (1993) Perspectives in diabetes: lessons from the diabetes control and complications trial. *Diabetes* 42: 1549-1554
3. The DCCT Research Group (1990) Epidemiology of severe hypoglycemia in the Diabetes Control and Complications Trial. *Am J Med* 90: 450-459
4. The DCCT Research Group (1988) Weight gain associated with intensive therapy in the Diabetes Control and Complications Trial. *Diabetes Care* 11: 567-573
5. Carlson MG, Campbell PJ (1993) Intensive insulin therapy and weight gain in IDDM. *Diabetes* 42: 1700-1707

6. Tobin BW, Rajotte RV, Ryan EA, Finegood DT (1992) Secondary pathologic conditions in diabetes mellitus and the benefit of improved metabolic control achieved through pancreatic islet transplantation. *J Cardiopulmonary Rehab* 12: 399–402
7. Mauer SM, Sutherland DER, Steffes MW et al. (1974) Pancreatic islet transplantation: effects on the glomerular lesions of experimental diabetes in the rat. *Diabetes* 23: 748–753
8. Krupin T, Waltman SR, Scharp DW (1979) Ocular fluorophotometry in streptozotocin diabetes mellitus in the rat: effect of pancreatic islet isografts. *Invest Ophthalmol Vis Sci* 18: 1185–1190
9. Schmidt RE, Plurad SB, Olack BJ, Scharp DW (1983) The effect of pancreatic islet transplantation and insulin therapy on experimental diabetic autonomic neuropathy. *Diabetes* 32: 532–540
10. MacLean IM, Rajotte RV, Belcastro AN (1987) Insulin and islet cell transplants: effects on diabetic cardiac myofibril ATPase. *Am J Physiol* 252: E244–E247
11. Portis AJ, Warnock GL, Finegood DT, Belcastro AN, Rajotte RV (1990) Glucoregulatory response to moderate exercise in long-term islet cell autografted dogs. *Can J Physiol Pharmacol* 68: 1308–1312
12. Pipeleers-Marachal MA, Pipeleers DG, Cutler J, Lacy PE, Kipmish DM (1976) Metabolic and morphological studies in intraportal islet transplanted rats. *Diabetes* 25: 1041–1051
13. Trimble ER, Karakash F, Malaisse-Lagae F, Vassutine I, Orci L, Renold AE (1980) Effects of intraportal transplantation on the transplanted tissue and the recipient pancreas. I. Functional studies. *Diabetes* 29: 341–347
14. Rajotte RV, Thompson ABR (1987) Effect of diet on islet cell transplantation. *Diabetes Res* 4: 125–130
15. Tobin BW, Lewis JT, Chen Z, Finegood DT (1993) Insulin secretory function in relation to the transplanted islet mass in streptozotocin diabetic rats. *Diabetes* 42: 98–105
16. Ballinger WF, Lacey PE (1972) Transplantation of intact pancreatic islets in rats. *Surgery* 72: 175–186
17. Ricordi C, Gray DWR, Hering BJ et al. (1990) Islet isolation assessment in man and large animals. *Acta Diabetol Lat* 27: 185–195
18. Russ RD, Resta TC, Walker BR (1992) Pulmonary vasodilatory response to neurohypophyseal peptides in the rat. *J Appl Physiol* 73: 473–478
19. Hall CB, Lukaski HC, Marchello MJ (1989) Estimation of rat body composition using tetrapolar bioelectrical impedance analysis. *Nutr Rep Int* 39: 627–633
20. Hartsook EW, Hershberger TV (1963) A simplified method for sampling small animal carcasses for analysis. *Proc Soc Exp Biol Med* 113: 973–977
21. AOAC (1990) Official Methods of Analysis (15th ed.) Association of Official Analytical Chemists, Washington, DC
22. Heymsfield SB, Wang J, Heshka S, Kehayias JJ, Pierson RN (1989) Dual-photon absorptiometry: comparison of bone mineral and soft tissue mass measurements in vivo with established methods. *Am J Clin Nutr* 49: 1283–1289
23. Tobin BW, Lewis JT, Finegood DT (1993) Effects of pancreatic islet transplantation on plasma glucose and hyperphagia in diabetic rats. *FASEB J* 7: A91 (Abstract)
24. Wadden TA (1993) Treatment of obesity by moderate and severe caloric restriction: results of clinical research trials. *Ann Int Med* 119: 688–693
25. Walsh CH, Soler NG, James H et al. (1976) Studies in whole body potassium and whole body nitrogen in newly diagnosed diabetics. *Q J Med N S* 45: 295–301
26. Nair KS, Garrow JS, Ford C, Mahler RF, Halliday D (1983) Effect of poor diabetic control and obesity on whole body protein metabolism in man. *Diabetologia* 25: 400–403
27. Morgan HE, Jefferson LS, Wolpert EB, Rannels DE (1971) Regulation of protein synthesis in heart muscle. II. Effect of amino acid levels and insulin on ribosomal aggregation. *J Biol Chem* 246: 2163–2170
28. Jefferson LS, Li JB, Rannels SR (1977) Regulation by insulin of amino acid release and protein turnover in the perfused rat hemi-corpus. *J Biol Chem* 252: 1476–1483
29. Peavy DE, Taylor JM, Jefferson LS (1978) Correlation of albumin production rates and mRNA levels in livers of normal, diabetic and insulin-treated diabetic rats. *Proc Nat Acad Sci USA* 75: 5879–5883
30. Levin ME, Boisseau VC, Avioli LV (1976) Effects of diabetes mellitus on bone mass in juvenile and adult-onset diabetes. *N Engl J Med* 294: 241–245
31. Hough FS (1987) Alterations of bone and mineral metabolism in diabetes mellitus. *S Afr Med J* 72: 120–126
32. Goodman WG, Hori MT (1984) Diminished bone formation in experimental diabetes: relationship to osteoid maturation and mineralization. *Diabetes* 33: 825–831
33. Bouillon R (1991) Diabetic bone disease. *Calcif Tis Int* 49: 155–160