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*Original Contribution***CONTROL OF INSULIN SECRETION AND GLUCOSE HOMEOSTASIS IN EXERCISING DIABETIC RATS WITH INTRASPLENIC OR KIDNEY SUBCAPSULAR ISLET GRAFTS**HARMINA HOUWING,* LUCHIENA G. HILBRANDS,* PAUL T.R. VAN SUYLICHEM,† JAN E. BRUGGINK,*
ANTON B. STEFFENS,* AND JAN H. STRUBBE*¹

*Department of Animal Physiology, University of Groningen, P.O. Box 14, 9750 AA Haren, †Department of Surgery, University of Groningen, Bloemsingel 1, 9713 BZ Groningen, The Netherlands

Abstract — This study was designed 1) to investigate mechanisms of insulin secretion during exercise after transplantation of islets in the spleen and under the kidney capsule, and 2) to compare these organs as transplantation site regarding an adequate portal or systemic delivery of insulin and glucose homeostasis during exercise. Diabetic rats were provided with 5 μ L isogenic islet tissue in the spleen or under the kidney capsule, which results in normoglycemia, and were submitted to a swimming test. Portal plasma insulin levels were higher than simultaneously sampled systemic insulin levels in the control and in the intrasplenic islet grafted group, but not in the kidney subcapsular islet-grafted group. Plasma portal and systemic insulin levels decreased, and glucose levels increased during exercise in all groups. The exercise-induced increase in levels of catecholamines was larger in systemic than in portal plasma, suggesting catecholamine extraction by the lungs or intestines. The experiments were repeated after removing of adrenal medulla, resulting in nondetectable or very low plasma adrenaline levels. Despite these low adrenaline levels, insulin levels decreased during exercise. The results indicate that 1) the exercise-induced reduction of insulin secretion is not mediated by circulating adrenaline, but is probably under control of the sympathetic nervous system, which could be the result of reinnervation of the transplanted islets. 2) Although a portal-systemic insulin gradient was absent in rats with kidney subcapsular islet grafts, the absence of a difference in glucose homeostasis during exercise between the sites revealed that all investigated sites are preferential to transplant islets. © 1997 Elsevier Science Inc.

Keywords — Islet transplantation; Spleen; Kidney capsule; Exercise; Reinnervation; Portal insulin delivery.

INTRODUCTION

Successful clinical islet transplantations in the liver of diabetic humans have been reported and summarized

recently (8,18). The liver was used as transplantation site because the operation procedure is relatively easy. Several studies in animals have been performed to find the best implantation sites with regard to duration of graft function and metabolic function (20,23,25,26,38,39). The renal subcapsular space was reported to offer the best conditions for growth of the islets, which results in a longer functioning period (25). Transplantation of islets into the spleen might be preferable because normoglycemia is achieved with lower systemic insulin levels due to the portal venous insulin delivery (26,38,39). Portal insulin delivery might, therefore, prevent peripheral insulin resistance due to high systemic insulin levels (2). Similar results were obtained in other studies in which insulin was delivered in either the portal or the systemic circulation (1,4,11,30). In many studies, baseline systemic insulin levels or systemic insulin levels after glucose administration were compared following islet transplantation in organs with either portal or systemic insulin delivery (26,38,39). However, most of these studies were performed under rest conditions, so that comparison between transplantation sites and portal or systemic insulin delivery could not be made under conditions that reflect other normal activities. For instance, the effect of exercise on insulin secretion and glucose homeostasis under these conditions is not known so far.

A reduction of insulin secretion and an enhancement of glucagon secretion during exercise is important because it increases hepatic glucose production (10,43). An increased sympathoadrenal activity is an important factor to decrease the insulin and to increase the glucagon

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¹Correspondence should be addressed to Dr. J.H. Strubbe,

Department of Animal Physiology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands.

secretion (10,32). An adequate function of these physiological mechanisms during exercise may therefore be important for energy metabolism. However, because the innervation of the endocrine pancreas may be absent or inadequate after islet transplantation, energy metabolism may be affected. Therefore, the objectives of the present study were 1) to investigate the mechanism responsible for a possible exercise-induced reduction of insulin secretion after transplantation of islets in the spleen and under the kidney capsule, and 2) to compare the portal insulin drainage of intrasplenic islet grafts and the systemic drainage of islets under the kidney capsule with regard to glycaemia during exercise. We used control rats and streptozotocin (STZ)-induced diabetic rats provided with isogenic islet grafts in the spleen or under the kidney capsule. A swimming exercise experiment was performed, and both systemic and portal blood was sampled to obtain the most appropriate assessment of islet function and of influences on the liver. In particular, in animals with intrasplenic islet grafts, peripheral data only might not be adequate to determine insulin outflow (27). The adrenal medullas were removed to test the inhibitory effects of circulating adrenaline on insulin secretion and the exercise experiment was repeated.

MATERIALS AND METHODS

Animals and Experimental Design

Male Albino Oxford (AO/G) rats of 338 ± 7 g were obtained from the Central Animal Laboratory of the University of Groningen, The Netherlands. These animals were maintained at a 12:12 h light:dark cycle at a room temperature of about 20°C in Perspex cages. They were fed with standard lab chow (Hope Farms, Woerden, The Netherlands) and had free access to water. Diabetes was induced with 70 mg/kg b.wt. STZ (Zanozar, Upjohn Company, Kalamazoo, MI) injected via the penis vein. This resulted in loss of body weight, glucosuria, increased water intake, and blood glucose levels of more than 20 mmol/L. Then, 2–3 wk after the diabetes induction, islet tissue isolated from isogenic islet donors with a body weight of 350–400 g was transplanted into the spleen or under the kidney capsule according to the procedure shortly described in the next section.

After islet transplantation and return of normoglycemia, these rats and untreated control rats were provided with a heart catheter via the jugular vein allowing stress-free sampling of systemic blood (33). The heparinized silicon heart catheter (11 cm, i.d. 0.51 mm, o.d. 0.94 mm; Dow Corning, Midland, MI) was drawn under the skin to the skull where it was attached. In addition animals were provided with a heparinized silicon catheter (22 cm, i.d. 0.51 mm, o.d. 0.94 mm; Dow Corning) to sample blood from the portal vein. The surgery technique

was described in detail previously (36). The tip of the catheter was situated in the main stream of the portal vein, whereas the other end was drawn and attached to the skull. The catheters were filled with 50% (W/V) polyvinylpyrrolidone in a heparin solution (500 IU/mL saline). At least 1 h before the start of the experiments, the catheters were connected to a polyethylene blood sampling tube, filled with saline. Swimming exercise experiments were performed after animals had regained their preoperative body weight (3 wk after transplantation). After these experiments, the adrenal medulla was removed by extirpation in all animals to exclude influence of adrenaline on metabolic processes and hormone secretions. After an incision in the adrenal cortex, the adrenal medulla was popped out by slight pressure using a pair of tweezers. Using this method, it is possible to remove the inner part of the adrenal as a whole, without serious damage of the cortex. None of the adrenalectomized rats drank the saline solution that was offered in addition to the normal water, indicating that mineralocorticoids were still available. The swimming exercise experiments were repeated at least 1 wk after adrenalectomy.

The STZ injections and islet transplantations were performed under ether anesthesia. Both the jugular vein and portal vein cannulations and the adrenalectomies were performed under halothane anesthesia.

Islet Isolation and Transplantation

The rat islet isolation method as used in our laboratory has been described previously (40). Briefly, the pancreas was distended by infusing 10 mL Krebs' Ringer solution containing 25 mmol/L HEPES and 10% bovine serum albumin into the pancreatic duct. The pancreas was then excised and cut into small pieces with a pair of scissors. A two-stage collagenase (Sigma type XI, 2200 U/mg, Sigma, St. Louis, MO) digestion was performed at 37°C at concentrations of 1.2 and 0.7 mg/mL, respectively. Islets were separated from the exocrine tissue using a discontinuous dextran gradient (Sigma industrial grade, molecular weight 70,000). Further purification of the islets was obtained by handpicking to eliminate nonseparated lymph nodes and vascular and ductal tissue from the islet grafts. Islets were identified with the aid of a dissection microscope (Bausch and Lomb 31-28-06) and a fluorescent lamp (Bausch and Lomb 31-33-66). With this illumination, rat islets appear as distinct ochreous bodies, whereas lymph nodes and exocrine tissue are grey. The reliability of this method has been confirmed by histology and dithizone staining.

The total islet volume obtained was determined by measuring the islet diameters, expressed as the mean of two axes, of islets in a 5% aliquot of the islet suspension.

By assuming the islets to be perfect spheres, islet volume was calculated. Grafts of 5 μL endocrine tissue were prepared by taking an appropriate portion of the total islet suspension. This graft volume is about 50% of the content of a normal adult pancreas, as determined by measuring insulin content of a volume of 10 μL (38). Transplantation into the spleen was performed immediately after the islet isolation by direct puncture with a 23-gauge butterfly needle in the splenic parenchyma. During infusion into the spleen, the splenic pedicle was manually occluded to reduce possible islet loss to the liver (9). Transplantation under the kidney capsule was performed at the upper pole by carefully expelling the islets from a polyethylene tube introduced at the lower pole of the kidney. After the transplantation was completed, the syringes, butterfly needles, and the polyethylene tubes were examined to confirm that all islets had been transplanted.

Exercise Experiments

Exercise against a flow of 0.22 m/s was performed in a swimming pool (length 3.0 m; width 0.4 m; depth 0.9 m) containing water at $33 \pm 2^\circ\text{C}$. Blood samples with volumes of 0.4 to 0.6 mL were taken simultaneously from both the heart catheter and the portal vein catheter for determination of glucose in whole blood or insulin, adrenaline, and noradrenaline in plasma. After sampling, the withdrawn volume of blood was immediately replaced by an equal volume of heparinized donor blood. The samples were taken in the home cage ($t = -23$ and -13 min), at the waiting platform ($t = -11.5$ and -3 min), during lowering of this platform ($t = -1$), during swimming ($t = 1, 5, 10$, and 15 min), and at the resting platform ($t = 19, 24, 29$, and 39 min). The construction of this swimming pool as well as the experimental procedure was described previously by Scheurink et al. (31,32).

Chemical Determinations

The blood samples were immediately transferred to chilled centrifuge tubes containing 10 μL heparin solution (500 IU/mL) as anticoagulant and 0.01% EDTA as antioxidant.

Glucose concentrations were measured in 50 μL whole blood with the Hoffman's ferricyanide method in a Technicon autoanalyzer TMII. The remaining blood was centrifuged for 10 min at 5000 rpm at 4°C . For the assay of catecholamines, 100 μL plasma was stored at -80°C . The remaining plasma was stored at -20°C for the determination of insulin concentrations.

Catecholamine concentrations were analyzed in 90 μL plasma by high-pressure liquid chromatography in combination with electrochemical detection as previously

described (31). The lower detection limit for adrenaline was 54.6 pmol/L and 0.03 nmol/L for noradrenaline.

Insulin concentrations were measured in duplicate in a radioimmunoassay (RIA) using Guinea pig serum as antiserum (M8309, NOVO, Copenhagen). The bound and free ^{125}I -labeled insulin were separated by means of a polyethylene glycol solution as described by Henquin et al. (13).

Statistical Analysis

Data are expressed as means $\pm$ SEM and were evaluated using multivariate analysis of variance for repeated measures (MANOVA of SPSS/PC+). The Mann-Whitney *U*-test was used for testing the source of variation between groups. The Wilcoxon's matched-pairs signed-ranks test was used for testing differences within groups. Differences with a value of $p < 0.05$ were considered significant.

RESULTS

Control Group

The results of the exercise experiments in the control group are presented in Fig. 1. Blood glucose levels were similar in the portal vein and jugular vein, and increased due to exercise. Portal plasma insulin concentrations were higher than systemic plasma insulin concentrations before and after exercise ($p < 0.05$), but not during exercise ($t = 5$, and 10). Both portal and systemic insulin levels decreased during exercise. Portal insulin fluctuated more before, during, and after exercise than insulin in the jugular vein. Plasma adrenaline levels were lower in the portal circulation than in the systemic circulation ($p < 0.05$) and increased to much higher levels in the systemic circulation than in the portal circulation when the starting platform was lowered into the water. Baseline levels of plasma noradrenaline were similar in both sample sites and increased more in the systemic than in the portal circulation during exercise ($p < 0.005$).

The results obtained after adrenalectomy in the control group are depicted in Fig. 2. Basal and exercise-induced glucose levels were higher in the portal vein of control rats than in the jugular vein of control rats at most time points ($p < 0.05$). Adrenalectomy resulted in lower increases in glucose levels during and after swimming, when compared to the situation before adrenalectomy ($p < 0.05$; see Fig. 1). Adrenalectomy reduced portal plasma insulin levels to similar levels as in the jugular vein. The exercise-induced reduction in plasma insulin levels was still present. As expected, plasma adrenaline levels were not detectable in the adrenalectomized control group. The exercise-induced increase in plasma noradrenaline levels ($p <$

Fig. 1. Effect of exercise on concentrations of blood glucose, plasma insulin, adrenaline, and noradrenaline before, during, and after swimming in the portal vein (●) and in the jugular vein (○) in control rats ($n = 8$). All data are expressed as mean $\pm$ SEM.

0.005) was similar to that in the control group before adrenalectomy.

Intrasplenic Islet-Grafted Group

The mean blood glucose levels before intrasplenic islet transplantation were 23.1 ± 1.4 mmol/L. Transplantation normalized blood glucose levels within 5.5 ± 1.0 days (Table 1). In Fig. 3, the results of the exercise experiments in the diabetic animals with islet grafts in the spleen are presented. The blood glucose levels increased throughout the experiment ($p < 0.05$), were similar in the portal and systemic circulation, and were not different from the concentrations in the control group.

Fig. 2. Effect of exercise on concentrations of blood glucose, plasma insulin, adrenaline, and noradrenaline before, during, and after swimming in the portal vein (●) and in the jugular vein (○) in adrenomedullated control rats ($n = 7$). All data are expressed as mean $\pm$ SEM.

Plasma insulin levels decreased during exercise and, as in control animals, portal insulin levels fluctuated more and were higher than systemic insulin levels at most time points ($p < 0.05$). Plasma adrenaline levels were not different from those in the control group and were much higher in the systemic circulation than in the portal circulation ($p < 0.05$). Plasma noradrenaline levels increased during exercise ($p < 0.05$) and both the systemic and the portal noradrenaline were lower than in control animals ($p < 0.05$).

The effects of exercise on blood components after adrenalectomy, which led to nondetectable adrenaline levels and lower exercise-induced increases in

Fig. 3. Effect of exercise on concentrations of blood glucose, plasma insulin, adrenaline, and noradrenaline before, during, and after swimming in the portal vein (●) and in the jugular vein (○) in rats provided with intrasplenic islet grafts ($n = 6$). All data are expressed as mean $\pm$ SEM.

Fig. 4. Effect of exercise on concentrations of blood glucose, plasma insulin, adrenaline, and noradrenaline before, during, and after swimming in the portal vein (●) and in the jugular vein (○) in rats provided with intrasplenic islet grafts after adrenalectomy ($n = 8$). All data are expressed as mean $\pm$ SEM.

Table 1. Body weight and systemic blood glucose levels after STZ injection (mean $\pm$ SEM), and time period in days needed to return to the normoglycemic state after islet transplantation in the spleen or under the kidney capsule.

	Transplantation Site	
	Spleen	Kidney Capsule
Body weight before STZ (g)	323 $\pm$ 18	318 $\pm$ 9
Body weight after STZ (g)	268 $\pm$ 9	273 $\pm$ 10
Glucose levels after STZ (mmol/l)	23.1 $\pm$ 1.4	22.8 $\pm$ 1.3
Normoglycemia reached after day	5.5 $\pm$ 1.0	6.5 $\pm$ 0.2

blood glucose levels ($p < 0.05$), are presented in Fig. 4. No significant differences were observed between the blood glucose levels in the portal and systemic circulation or between the blood glucose levels in the adrenalectomized intrasplenic islet-grafted group and the adrenalectomized control group. Portal plasma insulin levels were higher than the systemic plasma insulin levels before exercise ($p < 0.05$), but not during exercise. This situation is similar to that in adrenalectomized control animals (see Fig. 2). In addition, plasma insulin levels dropped during exercise. Plasma adrenaline levels were hardly detectable, and plasma noradrenaline

Fig. 5. Effect of exercise on concentrations of blood glucose, plasma insulin, adrenaline, and noradrenaline before, during, and after swimming in the portal vein (●) and in the jugular vein (○) in rats provided with islet grafts under the kidney capsule ($n = 6$). All data are expressed as mean $\pm$ SEM.

levels during exercise were not significantly higher than those before adrenalectomy and were not different from those in adrenalectomized control animals.

Kidney Subcapsular Space Islet-Grafted Group

Before transplantation of islets under the kidney capsule, blood glucose levels were 22.8 ± 1.3 mmol/L. Transplantation reversed the hyperglycemic state within 6.5 days (Table 1). Levels of blood components during exercise in the diabetic group with islet grafts under the kidney capsule are presented in Fig. 5. Blood glucose levels were similar to those in the control group and in

Fig. 6. Effect of exercise on concentrations of blood glucose, plasma insulin, adrenaline, and noradrenaline before, during, and after swimming in the portal vein (●) and in the jugular vein (○) in rats provided with islet grafts under the kidney capsule after adrenalectomy ($n = 3$). All data are expressed as mean $\pm$ SEM.

the group with intrasplenic islet grafts, and increased during and after exercise ($p < 0.05$). No difference was observed between the portal and systemic blood glucose levels. Unlike the situation in control and in intrasplenic islet-grafted animals, in which portal insulin levels were higher than systemic insulin levels, portal and systemic insulin levels were similar in the group with islet grafts under the kidney capsule. The fluctuations in systemic insulin levels were more pronounced in this group than in the other groups. Insulin levels slightly decreased in both the portal and systemic circulation during exercise.

Plasma adrenaline levels increased in the portal, but much more in the systemic circulation when the starting platform was lowered into the water ($p < 0.005$), and did not differ from the other groups with intact adrenal medullas. Basal plasma and exercise-induced increments ($p < 0.005$) in plasma noradrenaline concentrations were similar to those in the intrasplenic islet-grafted rats, but were lower than in control rats ($p < 0.05$).

The results after adrenalectomy are presented in Fig. 6. Blood glucose levels during and after exercise were lower after adrenalectomy than before adrenalectomy ($p < 0.05$) and slightly lower than in the other adrenalectomized groups. As before adrenalectomy, insulin levels were similar in the portal and systemic circulation and decreased slightly during exercise. In the animals with islet grafts under the kidney capsule, adrenalectomy resulted in very low adrenaline levels. Plasma noradrenaline levels were not significantly lower than before adrenalectomy, but they were lower than in other adrenalectomized groups.

DISCUSSION

The results of the present study in which the spleen and the kidney subcapsular space were compared as implantation sites for islets of Langerhans indicated that insulin secretion was reduced during exercise in all groups both before and after adrenalectomy. There is evidence that the secretion of insulin is reduced during exercise due to α_2 -adrenergic stimulation of the beta cell in the pancreatic islets under normal conditions (32). Noradrenaline from the noradrenergic nerve endings is the predominant factor to reduce insulin secretion during exercise (14,16,19,32). Because the islets of Langerhans become denervated during the isolation procedure, the drop in insulin levels during swimming in islet-transplanted rats can be caused by either circulating catecholamines or by catecholamines released from newly ingrown noradrenergic nerve endings into the transplanted islets. The reduction of insulin secretion remains present after adrenalectomy, and an infusion of adrenaline and noradrenaline in physiological concentrations did not reduce insulin secretion in intraportal islet grafted rats (17). Thus, circulating catecholamines are probably not responsible for the reduction in insulin secretion. Because there is histological evidence that islet grafts under the kidney capsule and in the spleen become reinnervated with noradrenergic nerve fibres (17,21,22,24,28), it is very probable that newly ingrown noradrenergic nerve fibers into these islet grafts, as in rats with transplanted islets in the liver (16), are responsible for the reduction in insulin release.

A similar portal-systemic insulin gradient was ob-

served in the animals with intrasplenic islet grafts as in the control animals. This indicates that most of the transplanted islets remained in the implantation sites, although some of them might have migrated from the splenic pulp into the liver (39). The drop in insulin levels in the portal vein of control and intrasplenic islet-grafted diabetic rats is stronger than in the jugular vein. It is possible that insulin extraction by the liver during exercise is reduced, as previously suggested (5,12). However, it is more probable that the reduction in insulin secretion is more pronounced in the portal vein due to the smaller dilution in the blood volume flowing through the portal vein. Positive and negative changes in insulin concentration will, thus, be less pronounced in the higher volume of the systemic circulation with more residual insulin. Similar results were also obtained in dogs bearing an intrasplenic islet graft after oral meal tests (27). In the group with islets under the kidney capsule, insulin levels fluctuated in a similar way in both systemic and portal plasma. Portal insulin was more suppressed during swimming in adrenalectomized control rats and in adrenalectomized rats with intrasplenic islet grafts, which must be due to a stronger reduction in secretion of insulin. The mechanism leading to the stronger reduction in insulin secretion after adrenalectomy is not clear, but might be caused by the lower glucose levels during exercise.

The overall comparison between the portal and systemic circulation showed that blood glucose levels in the portal vein were mostly not significantly higher than in the systemic circulation. This is in contrast to earlier observations in Wistar rats, and might be dependent on the nutritional state (35). Plasma adrenaline levels in the systemic circulation were much higher than those in the portal circulation. Apparently, adrenaline, which stimulates hepatic glucose output, reaches the liver mainly by hepatic arterial supply. These much lower portal adrenaline levels are possibly the consequence of extraction by the lungs and several organs drained by the portal system, like the intestines, the spleen, and the pancreas. Adrenaline, acting on the pancreas, has minor influences on insulin secretion during exercise (16,32), but has impact on glucagon secretion (10,37). The fact that noradrenaline concentrations were lower in the portal circulation than in the systemic circulation suggests uptake of the noradrenaline supplied by the systemic circulation in the lungs and in organs connected with the portal circulation, including the intestines, the spleen, and the pancreas, as also reported for other tissues (6). Intravenous infusions of noradrenaline in concentrations that mimic exercise-induced plasma noradrenaline concentrations have no effect on insulin release or blood glucose levels (16,34). Circulating noradrenaline might affect metabolism and lipolysis in adipose tissue (32) or

it may affect blood flow through the intestines (3,41). This means that hepatic glucose output is mainly stimulated by noradrenergic neural influences and by hepatic arterial supply of adrenaline.

Glucose homeostasis during exercise was similar in both groups with islet grafts and did not differ from exercise-induced glucose homeostasis in control animals. Normal glycemic responses to exercise were also reported in dogs bearing intrasplenic islet grafts (29). Previous results, obtained from similar exercise experiments in diabetic rats with islets injected into the portal vein, demonstrated similar glycemic adaptations in the systemic circulation (15,16). The glycemic adaptations in exercise were accompanied by similar systemic plasma insulin levels in all groups, as also reported in nonexercising rats transplanted with fetal pancreatic islets under the kidney capsule or into the splenic pulp (23). Other studies reported higher baseline peripheral insulin levels following systemic insulin delivery after transposition of the pancreatic venous drainage to the systemic circulation before (2) or after transplantation of endocrine pancreatic tissue (7,26), or after islet transplantation under the kidney capsule (38,39). Exercise-induced noradrenaline outflow before adrenodemedullation was lower in both transplanted groups than in the control group, but was not different from controls in the intrasplenic islet-grafted groups after removal of the adrenal medulla. The reason for these differences in plasma noradrenaline levels remains unclear, but is not due to different exercise intensities.

The lower exercise-induced noradrenaline levels in both islet-transplanted groups and the absent portal-peripheral insulin gradients in the animals with islet grafts under the kidney capsule have minor influences on blood glucose levels during exercise. This means that with regard to glucose metabolism and insulin secretion, none of the investigated sites is preferential to transplant islets. Previous results in animals with islets transplanted into the portal vein showed that exercise-induced systemic blood glucose and plasma insulin, adrenaline, and noradrenaline levels were similar to those in controls (15,16). Therefore, and because this site is already reported to be successful in clinical experiments (8,18), the liver might still be a possible preferential site to transplant islets. However, other results show that less insulin is required to normalize glucose levels after tolerance tests in intrasplenic islet-grafted rats (39), and long-term survival in intrasplenic islet-grafted dogs is better (42), making the spleen a preferential site. Long-term studies and measurements of other parameters may show in the future whether higher portal insulin levels favor a better control of metabolic physiological processes during exercise due to a more integrated and direct influence on liver cells.

In summary, the present study therefore indicates that 1) exercise-induced insulin secretion in diabetic rats provided with islet grafts in the spleen and under the kidney capsule is inhibited, which is probably the result of reinnervation of the islets by the sympathetic nervous system, and that 2) because there are no marked differences in insulin secretion and glycaemia during exercise, until now all sites are preferential to transplant islets of Langerhans.

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