Metabolic Control in Diabetic Dogs Treated with Pancreatic Autotransplants and Insulin Pumps

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SUMMARY

Fasting metabolite and hormonal levels were studied prospectively in pancreatectomized dogs who had received grafts of their own pancreas. The results were compared with similarly diabetic animals who received exogenous insulin pumped intravenously either peripherally or portally. All animals were studied for 48–91 wk after pancreatectomy.

In the autotransplanted animals, the fasting levels of glucose, lactate, pyruvate, alanine, pancreatic glucagon, insulin, gastric inhibitory peptide, and pancreatic polypeptide were all abnormal. In the peripherally infused animals, the fasting levels of glucose, pyruvate, alanine, free fatty acids, and insulin were also abnormal. In the portally infused animals, pyruvate, alanine, gastric inhibitory peptide, gastrin, and pancreatic polypeptide were abnormal.

These results suggest that the portal route of insulin delivery may be necessary if fasting metabolite and hormonal levels are to approximate normal most closely whether exogenous intravenous insulin is replaced by implanted pumps or endogenous insulin is replaced by pancreatic transplants. DIABETES 1986; 35:97–100.

Pancreatic transplantation corrects the insulin deficiency in human[1] as well as experimental[2–4] diabetes. Glycemia is normalized and injections of insulin are eliminated. Similar results can also be obtained when exogenous insulin is pumped intravenously.[4–6] Transplanted glands are denervated and usually drain into the peripheral rather than portal circulation. It is not known whether these differences in endogenous insulin delivery result in metabolic abnormalities or whether similar abnormalities occur when exogenous insulin is delivered intravenously either portally or peripherally by a pump. A comparison of the two methods of diabetes treatment in an animal model has not been done.

The research reported in this article therefore explored the degree to which fasting metabolism was normalized in pancreatectomized dogs receiving grafts of their own pancreas and compared these findings to what was observed in similarly diabetic dogs receiving exogenous insulin pumped intravenously either peripherally or portally. For reference, nonpancreatectomized animals were also studied prospectively as normal controls.

MATERIALS AND METHODS

Animals. Eighteen dogs of mixed breeds were used in these studies. All were fed from 9 to 10 a.m. with a 620-g mixed meal consisting of 260 g dry food (Master Premium Dinner, Maple Leaf Mills, Toronto, Ontario, Canada) and 360 g soft meal (Meat Mix, Derby Pet Foods, Toronto, Ontario, Canada). In addition, each pancreatectomized dog received a total of 14–20 capsules of digestive enzymes (Cotazym, Organon Ltd., Montreal, P.Q., Canada) mixed with the meal to compensate for the surgically induced pancreatic exocrine deficiency. The pancreatectomized animals had normal stools and maintained their body weights. The daily light/dark cycle was 14/10 h and water was available ad libitum.

Eleven dogs weighing 12–23 kg were pancreatectomized as previously described.[6] In six, two silicone rubber catheters were placed as detailed.[7] Permanent silicone rubber catheters were placed: (1) for blood sampling, into an external jugular vein in all dogs, and (2) for exogenous insulin infusion, into the same vein in six dogs. For portally directed infusions, the portal vein was accessed via the splenic vein; for peripheral infusions, the inferior vena cava was accessed directly. Catheters were tunneled beneath the skin and exited on the back. In the remaining five animals a total pancreatectomy was also carried out, but care was taken to preserve

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the pancreatic branches of the splenic vessels. The body and tail of the pancreas, based on these vessels, were immediately transplanted to the lower abdomen. The artery and vein were anastomosed end-to-side to the aorta and to the inferior vena cava in four animals and to the iliac vessels in the other. Because thrombosis of the splenic artery occurred frequently, a distal A-V fistula between the splenic vessels[8] was used in two animals and an arterial jump graft (both proximal and distal ends of the splenic artery) anastomosed end-to-side to the host artery[9] was used in two animals. In every case, the pancreatic duct was left open to drain into the peritoneal cavity after the method of Kyriakides.[10]

All animals were followed for periods ranging from 48 to 91 wk. After this and other metabolic tests were completed, the pancreatic graft was either removed by operation or the insulin pump was stopped. Twenty-four hours later, all animals (except two that died overnight before blood glucose levels could be taken) were diabetic as evidenced by hyperglycemia >400 mg/dl and glycosuria.

Finally, seven healthy dogs weighing 12–14 kg received only the permanent jugular catheter for blood sampling and were sampled prospectively for similar periods of time to establish reference values in normal controls.

Pumps. Insulin solutions were delivered to the infused dogs from a refillable reservoir using a miniature peristaltic pump[8] and a battery-powered flow-rate controller.[9] The portable insulin delivery system weighed 650 g. In the postprandial period, a preprogrammed 3-pulse waveform was used whereby insulin infusion was accelerated above basal rates for time durations in keeping with reducing the postprandial glycemic excursion to approximate the normal response without hypoglycemia, similar to methods previously detailed.[5,10] The average waveform included a first pulse (1.85 mU/kg/min for 240 min), a second pulse (1.01 mU/kg/min for 120 min), and a third pulse (0.65 mU/kg/min for 120 min). Identical infusions were used portally and peripherally.

Insulin. Insulin solutions for the infused groups were as previously detailed.[5,6,11] These insulin solutions were introduced into the reservoirs carried on the dogs, and refilled periodically (approximately every 3–5 days) through integral bacterial filters (Millex, GS 0.22-μm filter unit, Millipore Corporation, Bedford, Massachusetts).

Blood samples. Approximately twice a month, fasting blood samples (7–5 ml) were taken at about 9 a.m. either through the indwelling jugular catheter or via direct venipuncture. Samples were aliquoted as follows: (1) 1.5 ml blood into microtubes containing 0.03 ml heparin (1000 IU/ml) for glucose and insulin; (2) 2 ml blood into ice-cold glass tubes containing an equal volume of 10% perchloric acid, for subsequent intermediary metabolite assays (lactate, pyruvate, β-hydroxybutyrate, and alanine); (3) 2 ml blood into glass tubes containing 0.2 ml EDTA and Trasylol for pancreatic glucagon and free fatty acid assays; and (4) 2 ml blood into similarly prepared glass tubes for determination of pancreatic polypeptide, gastrin, gastric inhibitory peptide, and entero-glucagon. Samples were kept on ice and within 30 min were spun in a refrigerated centrifuge, and the supernatant separated and stored at −20°C until assay.

Analytical methods. Plasma glucose was measured enzymatically using a Beckman Glucose Analyzer II (Beckman Instruments Inc., Fullerton, California). Plasma hormone concentrations were determined by radioimmunoassay utilizing techniques published in detail previously for gastrin,[12] gastric inhibitory peptide,[13] insulin,[5,6] pancreatic polypeptide,[14] and entero-glucagon.[13] Plasma entero-glucagon (glucagon-like immunoreactivity of intestinal origin) was derived by subtraction of values obtained using an antibody specific for pancreatic glucagon (RCS5)[13] from values obtained using antibody K4023 (Novo Research Institute, Copenhagen, Denmark). The values are expressed as equivalents of glucagon.

Lactate, pyruvate, and alanine were analyzed by microfluorometric adaptations of standard enzymic methods, as previously described.[15] Plasma free fatty acids were determined by a radiochemical assay as previously detailed.[16]

Standard statistical methods were employed using either the paired *t*-test to assess the significance of changes from baseline values observed before the meal, or the unpaired *t*-test to compare at corresponding time points the mean responses following meals of the group infused with insulin to the similar control group of animals not infused with insulin. All results are presented as mean ± SEM.

RESULTS

Metabolites. Although our findings were globally similar to what has been reported previously, we elucidated subtle differences that heretofore have not been considered. First, the mean fasting plasma glucose (FPG) concentration in the group of transplanted animals was slightly but significantly higher than normal, as shown in Figure 1. It was, however, similar to the supranormal levels we observed in the peripherally infused animals. Only the portally infused dogs had FPG levels entirely similar to the normal controls. Most remarkably, all treatment methods resulted in day-to-day var-

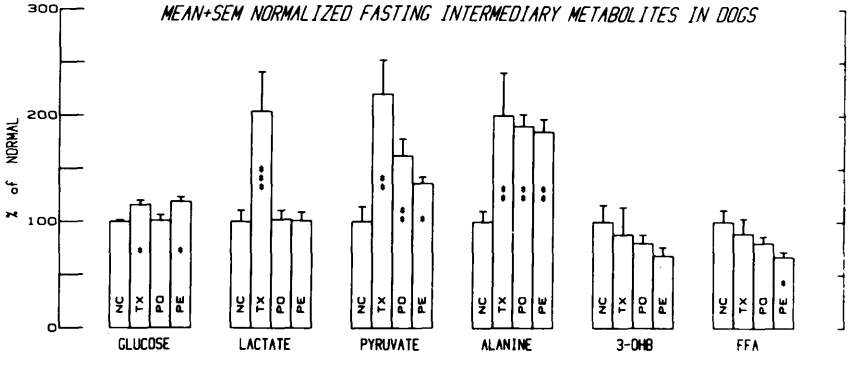

FIGURE 1. Comparison of fasting intermediary metabolite levels in normal (NC) dogs, in pancreas autotransplanted (TX) animals, and in pancreatectomized dogs receiving exogenous insulin infused either portally (PO) or peripherally (PE). All data expressed as mean ± SEM relative to NC, the latter normalized to 100%. 3-OHB, 3-hydoxybutyrate; FFA, free fatty acids.

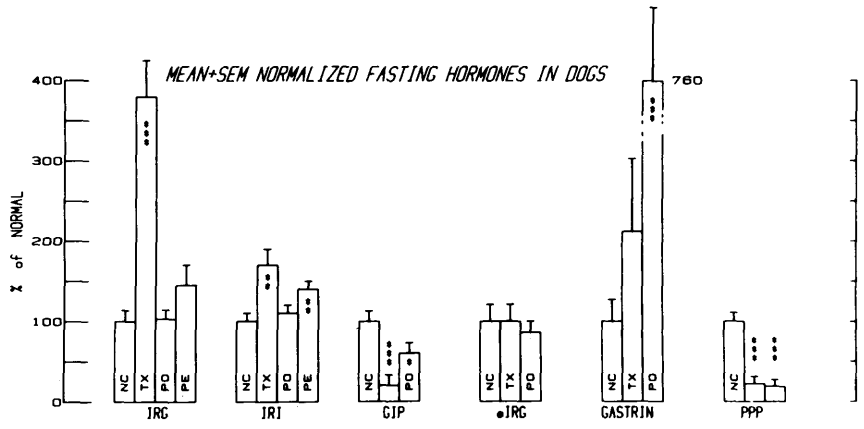

FIGURE 2. Comparison of fasting glucoregulatory and gastrointestinal hormone levels in animals as detailed in Figure 1. IRG, immunoreactive pancreatic glucagon; IRI, immunoreactive pancreatic insulin; GIP, gastric inhibitory peptide; eIRG, immunoreactive entero-glucagon; PPP, pancreatic polypeptide.

iations in FPG levels, which were significantly greater than normal with coefficients of variation 3–5 times normal.

In addition to the glycemic differences, there were significant differences in the levels of several key carbohydrates as well as fat intermediates of metabolism. Fasting lactate levels were elevated twofold above normal in transplanted animals, but were entirely normal in exogenously infused animals regardless of the route. Fasting pyruvate levels were elevated in all diabetic animals, but most notably in the transplanted and portally infused dogs. Fasting alanine levels were elevated at least twofold in all diabetic animals regardless of the method or route of insulin replacement. Free fatty acid levels were slightly below normal in all the diabetic animals but most notably in those who received exogenous insulin infusions peripherally. The levels of 3-hydroxybutyrate followed those of the free fatty acids in each group.

Hormones. Most remarkably, as shown in Figure 2, pancreatic immunoreactive glucagon (IRG) levels were profoundly elevated in the transplanted animals but entirely normal in the pancreatectomized dogs. Entero-glucagon (eIRG) was similar in all groups. Insulinemia was similar to normal only in the animals receiving exogenous porcine insulin portally. It was significantly elevated in both the peripherally infused and transplanted animals. Gastric inhibitory peptide was mildly reduced in the portally infused animals and reduced fivefold in the transplanted group. Gastrin was elevated twofold in the transplanted and almost eightfold in the portally infused animals. Finally, pancreatic polypeptide was reduced fivefold, to the detection limit of the assay not only in the pancreatectomized dogs, but also unexpectedly in the transplanted animals.

DISCUSSION

The present studies clearly reaffirmed that mean fasting plasma glucose concentrations can be readily controlled by pancreatic autotransplantation in dogs.[2] Notably, we documented a slight but significant elevation above normal. Whether in the autotransplanted animals this variability represented the peripheral route of endogenous insulin replacement or the aneural nature of the transplanted gland was not clarified. This interpretation was reinforced by the entirely similar fasting plasma glucose levels observed in the animals infused with exogenous insulin peripherally. Furthermore, when a similar group of animals received the same exogenous insulin by direct intraportal infusion, the mean fasting plasma glycemia dropped to the level found in the normal controls. These observations were consistent with the insulin levels observed in each group.

Together, these results suggest that the portal route of insulin delivery may be necessary if mean glycemia and insulinemia are to be normalized by either pancreatic transplantation or implanted pumps. Previous studies supported these notions[17,18] while others[19] failed to note a difference. The strength of the present studies lies in their long term, up to 91 wk, over which the animals were observed and therefore one can place confidence in the statistical interpretation of the results.

We have previously reported the ability of pumped exogenous insulin to normalize the fasting plasma glucose levels in dogs.[5] We emphasized that this desirable "normal" value was somewhat an artifact of the mean since it was associated with an abnormally large standard error, which we expressed as a coefficient of variation. In the present studies, this again occurred regardless of whether insulin was pumped peripherally or portally.

It was, however, rather unexpected that the autotransplanted animals should also exhibit large variations in fasting plasma glucose levels. These could not be attributed to intercurrent processes such as immune responses, rejection phenomena, or to side effects of other medications, e.g., for immunosuppression,[2] since these studies were conducted under ideal immunologic conditions. It is, however, noteworthy that even under these ideal conditions, graft survival did not endure beyond 2–3 yr.

In every animal, microscopic examination of the removed graft showed that the pancreatic acinar structure had completely atrophied. The remaining islet cells were often dispersed in long rows and surrounded by sheets of fibrous tissue (immunoperoxidase staining confirmed that these were islet cells). Acinar atrophy and fibrosis occurred even though the pancreatic duct had been left open to drain into the peritoneal cavity at the time of transplant. It was postulated that this fibrosis leads to vascular insufficiency and eventual islet failure in autotransplanted diabetic animals. We and others[9,20] have found that this occurred in dogs after 1–3 yr. It may be prevented if the duct is anastomosed to an epithelial surface, e.g., stomach, jejunum, ureter, or bladder.[20] In contrast, several of our diabetic dogs have received pumped exogenous insulin for well over 2 yr without detectable changes in insulin sensitivity or glycemic control.[21]

Can the observed abnormal variability in fasting metabolism be reduced? In transplanted animals, the source is not known. Beyond a year the autotransplanted dogs all showed signs of nutritional inadequacy in spite of enzyme replacement. This may have also contributed to the variability noted. Gradual loss of graft viability with exocrine necrosis may be prevented as mentioned above. Perhaps islet cell implants into the spleen will achieve this while advantageously routing insulin portally.[22] However, gland or islet cell pretreatment to minimize rejection or host immunosuppression are both processes that may reduce islet cell viability and lead to further metabolic instability. These processes as well as the architecture of islets and their distribution in the canine gland may have contributed to the profound hyperglucagonemia seen in the transplanted animals, as well as the unexpected fall in pancreatic polypeptide levels. The mechanisms behind the fall in gastric inhibitory peptide and the rise in gastrin are unclear.

In the exogenously infused dogs, the pumping systems were external and subjected to all the vagaries of animal experimentation, including lack of dietary compliance, physical activity, and mechanical and electrical failures as well as imperfect insulin solutions.[23] There thus appears to be adequate room for mechanical improvements. Implantable devices may overcome many of these problems and bring as a result significant reductions in the variability of fasting plasma glycemia. At present, it is not known whether this instability in fasting glycemia reflects the other intercurrent metabolic and hormonal abnormalities observed, nor is it known whether this degree of instability is important in terms of the long-term complications of diabetes.

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REFERENCES

[1] Sutherland, D. E. R., Najarian, J. S., Greenberg, B. Z., Senske, B. J., Anderson, G. E., Francis, R. S., and Goetz, F. C.: Vascularized segmental transplantation on the pancreas in insulin-dependent patients: hormonal and metabolic effects of an endocrine graft. Ann. Intern. Med. 1981; 95:521–37.

[2] Cutfield, R. G., Polonsky, K., Olson, L., Kyriakides, G., Miller, J., and Mintz, D. H.: Long-term follow-up of canine segmental pancreatic autografts. Diabetes 1985; 34:174–78.

[3] Brown, J., Clark, W. R., Molnar, I. G., and Mullen, Y. S.: Fetal pancreas transplantation for reversal of streptozotocin-induced diabetes in rats. Diabetes 1976; 25:56–64.

[4] Albisser, A. M., Nomura, M., and McPhedran, N. T.: Glycemic control in diabetic dogs treated with pancreatic autotransplants and insulin pumps. Diabetes Res. 1985; 2:145–49.

[5] Nomura, M., Zinman, B., Bahoric, A., Marliss, E. B., and Albisser, A. M.: Intravenous infusions of sulfated insulin normalize plasma glucose levels in pancreatectomized dogs. Diabetes 1983; 32:788–92.

[6] Goriya, Y., Bahoric, A., Marliss, E. B., Zinman, B., and Albisser, A. M.: Glycemic regulation using a programmed insulin delivery device. III. Long-term studies on diabetic dogs. Diabetes 1980; 28:558–64.

[7] Bahoric, A., Filler, R. M., Perlman, K., Jackman, W. S., and Albisser, A. M.: New devices and methods for long-term vascular access for blood sampling and insulin infusion. Diabetes Care 1980; 3:338–44.

[8] DuToit, D. F., Reece-Smith, H., McShane, P., Denton, T., and Morris, P. J.: A successful technique of segmental pancreatic autotransplantation in the dog. Transplantation 1981; 31:391–96.

[9] McPhedran, N. T., Attisha, R. A., Ross, S. A., and Eidt, P.: Pancreatic autotransplantation. Transplant. Proc. 1982; 14:709–13.

[10] Kyriakides, G. K., Rabinovitch, A., Mintz, D., Olson, L., Rapaport, F. T., and Miller, J.: Long-term study of vascularized free-draining intraperitoneal pancreatic segmental allografts in beagle dogs. J. Clin. Invest. 1981; 67:297–303.

[11] Goriya, Y., Bahoric, A., Marliss, E. B., Zinman, B., and Albisser, A. M.: Blood glucose control and insulin clearance in unrestrained diabetic dogs portally infused with a portable insulin delivery system. Diabetologia 1981; 19:452–57.

[12] Hall, K. E., Greenberg, G. R., El-Sharkaway, T. Y., and Diament, N. E.: Vagal control of migrating motor complex: related peaks in canine plasma motilin, pancreatic polypeptide and gastrin. Can. J. Physiol. Pharmacol. 1983; 61:1289–98.

[13] Greenberg, G. R., Wolman, S. L., Christofides, N. D., Bloom, S. R., and Jeejeebhoy, J. N.: Effect of total parenteral nutrition on gut hormone release in humans. Gastroenterology 1981; 80:988–93.

[14] Hall, K. E., Diament, N. E., El-Sharkaway, T. Y., and Greenberg, G. R.: Effect of pancreatic polypeptide on canine migrating motor complex and plasma motilin. Am. J. Physiol. 1983; 245:G178–85.

[15] Marliss, E. B., Murray, F. T., and Nakhooda, A. K.: Metabolic response to hypocaloric protein diets in obese man. J. Clin. Invest. 1978; 62:468–79.

[16] Ho, R. J.: Radiochemical assay of long-chain fatty acids using ^{63}Ni as tracer. Anal. Biochem. 1970; 36:105–13.

[17] Brown, J., Mullen, Y., Clark, W. R., Molnar, I. G., and Heininger, D.: Importance of hepatic portal circulation for insulin action in streptozotocin-diabetic rats transplanted with fetal pancreases. J. Clin. Invest. 1979; 64:1688–94.

[18] Stevenson, R. W., Parsons, J. A., and Alberti, K. G. M. M.: Effect of intraportal and peripheral insulin on glucose turnover and recycling in diabetic dogs. Am. J. Physiol. 1983; 244:E190–95.

[19] Botz, C. K., Leibel, B. S., Zingg, W., Gander, R. E., and Albisser, A. M.: Comparison of peripheral and portal routes of insulin infusion by a computer-controlled insulin infusion system (artificial endocrine pancreas). Diabetes 1976; 25:691–700.

[20] MacDonald, A. S., McKinnon, J. G., and Nalatajalian, D. A.: Pancreatic autotransplantation: duct drainage to bladder, duodenum, stomach, compared to duct ligation or free drainage into the peritoneal cavity. Transplant. Proc. 1982; 14:705–708.

[21] Albisser, A. M., Nomura, M., and Bahoric, A.: Pumped intravenous insulin in experimental diabetes. In press. Diabetes Res. 1986.

[22] Andersson, A.: Islet implantation normalizes hyperglycemia caused by streptozotocin-induced insulitis: experiments in mice. Lancet 1979; 1:581–84.

[23] Lougheed, W. D., Fischer, U., Perlman, K., and Albisser, A. M.: A physiological solvent for crystalline insulin. Diabetologia 1981; 20:51–53.