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## Puzzling About Partial Glucagon Responses to Hypoglycemia in Intrahepatic Islet Recipients: Missing Pieces



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Fifteen years ago, Shapiro et al. (1), at the University of Alberta in Edmonton, Canada, published an article in the *New England Journal of Medicine* that caused great excitement. They reported that seven consecutive recipients with type 1 diabetes who had received intrahepatic infusions of human islet allografts maintained normal levels of HbA<sub>1c</sub> for more than 1 year after transplant (4.4–14.9 months) without insulin treatment (1). They attributed their success to the infusion of a large total number of islets and the avoidance of glucocorticoid use for immunosuppression. The number of islet infusions depended on whether or not success was achieved with the preceding infusion. The average total mass of islets used was  $11,547 \pm 1,604$  islet equivalents/kg body weight (approximately 80% of that believed to be in a normal human pancreas). Glucocorticoids were avoided because of their known toxic effect on  $\beta$ -cells. Posttransplant C-peptide levels were stimulated twofold by mixed-meal tolerance tests. A subsequent publication by this group in 2002 reported that success appeared to be continuing as evidenced by sophisticated measures of  $\beta$ -cell function (2). However, by the fifth year of follow-up, these first blushes of prolonged success began to lose their glow. In 2005, Ryan et al. (3) reported that of 65 recipients, approximately 10% maintained insulin independence, 10% had failed, and approximately 80% remained C-peptide positive with normal or nearly normal HbA<sub>1c</sub> levels but were again using exogenous insulin treatment. Later trials verified this disappointing trend of falling short of prolonged success (4–7), although recent reports have generated a return to optimism (8–12).

Throughout these past 15 years, the emphasis on islet transplantation has been almost exclusively on  $\beta$ -cell function and avoidance of hyperglycemia. A consistent subtheme about benefits is that posttransplant recipients report a much lower frequency of hypoglycemic episodes, which can be very dangerous and are common in type 1 diabetes.

The most obvious explanation for fewer hypoglycemic episodes is that successful recipients stop using exogenous insulin. The more nuanced question is whether the intrahepatic transplanted islets themselves provide restoration of glucagon responses to hypoglycemia. In the first reported study of counterregulatory hormonal responses during hypoglycemic clamps in alloislet recipients, Paty et al. (13) raised questions about the intactness of  $\alpha$ -cell function. No significant increments in secretion of glucagon, epinephrine, or symptom awareness of hypoglycemia were observed posttransplant. Although disappointing, the finding of no glucagon response was not entirely surprising as Robertson and colleagues (14–16) had previously observed a lack of glucagon response to hypoglycemia, but not intravenous arginine, in intrahepatic autoislet recipients. Yet, glucagon responses were observed when islets were placed into the peritoneal cavity (16). Zhou et al. (17) later demonstrated that during hypoglycemia absent glucagon secretion from transplanted intrahepatic islets in normoglycemic streptozotocin-treated rodents could be restored by prolonged fasting to induce glycogen depletion and that this response could be made to disappear again by refeeding. It was concluded that increased glycogenolysis and free glucose flux within the liver had masked intrahepatic  $\alpha$ -cells from sensing systemic hypoglycemia. In 2005, Rickels et al. (18) detected no change above baseline glucagon levels during hypoglycemic clamps in recipients of intrahepatically transplanted alloislets, thus confirming the findings of Paty et al. (13).

In this issue of *Diabetes*, Rickels et al. (19) used stepped hyperinsulinemic–hypoglycemic and paired hyperinsulinemic–euglycemic clamps and observed increments in endogenous glucose production during hypoglycemic clamps in normoglycemic recipients of intrahepatic alloislet transplants. However, this response was not completely normal as the control subjects had a greater fall and later a greater rise in endogenous glucose production. This article also

demonstrated an absence of hypoglycemia during continuous glucose monitoring in the recipients, clearly reflecting the fact that they were no longer using exogenous insulin and thus no longer at risk for recurrent hypoglycemia and consequent hypoglycemic unawareness. However, following this logic, it is puzzling that the symptom response was not completely normal during the clamp studies. This may be related to the fact that the epinephrine response was less than that shown for control subjects. The authors also report that the partial restoration of epinephrine response began at 140 min and the partial glucagon response began at 160 min into the study. In view of the findings of other researchers (13–17) of no glucagon response from intrahepatic islets during hypoglycemia, this time lag between epinephrine and then glucagon secretion raises the question of whether the partial glucagon response observed by Rickels et al. (19) was caused by the earlier rise in epinephrine levels rather than hypoglycemia itself as epinephrine is known to be a direct agonist for glucagon release. One way to fill in the missing pieces of this puzzle is to perform these studies under conditions of adrenergic blockade.

Is it important to know whether the reported partial glucagon response (19) was stimulated by hypoglycemia or not? This issue is important because of previously published evidence that intrahepatic  $\alpha$ -cells do not respond to systemic hypoglycemia (13–18). In this regard, a recent article by Bellin et al. (20) demonstrated that during hypoglycemic clamps, glucagon responses were absent in recipients of intrahepatic autoislets, but normal glucagon responses were observed in recipients of both hepatic and nonhepatic autoislets. Interestingly, the recipients of islets in both hepatic plus nonhepatic sites had normal symptom responses to hypoglycemia, whereas the recipients of hepatic islets alone had absent symptoms. This once again indicates that  $\alpha$ -cells placed in the liver are not responsive to hypoglycemia, but they are responsive when placed in nonhepatic sites.

This commentary about the actual mechanisms responsible for the partial glucagon response to hypoglycemia in islet recipients may be considered to be an academic one. However, at a pragmatic level, it is important to consider using a transplant site that will allow  $\alpha$ -cells to robustly secrete glucagon to protect recipients from insulin-induced hypoglycemia. This is especially relevant because many intrahepatic islet recipients eventually use exogenous insulin and will be once again at risk for hypoglycemia. For this reason, it is recommended to infuse a substantial portion of islets (approximately 100,000) into a nonhepatic site when using the liver for islet transplantation (20).

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## References

- Shapiro AM, Lakey JR, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000;343:230–238
- Ryan EA, Lakey JR, Paty BW, et al. Successful islet transplantation: continued insulin reserve provides long-term glycemic control. *Diabetes* 2002;51:2148–2157
- Ryan EA, Paty BW, Senior PA, et al. Five-year follow-up after clinical islet transplantation. *Diabetes* 2005;54:2060–2069
- Oberholzer J, Triponez F, Mage R, et al. Human islet transplantation: lessons from 13 autologous and 13 allogeneic transplantations. *Transplantation* 2000;69:1115–1123
- Shapiro AM, Ricordi C, Hering BJ, et al. International trial of the Edmonton protocol for islet transplantation. *N Engl J Med* 2006;355:1318–1330
- Toso C, Baertschiger R, Morel P, et al.; GRAGIL group. Sequential kidney/ islet transplantation: efficacy and safety assessment of a steroid-free immunosuppression protocol. *Am J Transplant* 2006;6:1049–1058
- Alejandro R, Barton FB, Hering BJ, Wease S; Collaborative Islet Transplant Registry Investigators. 2008 update from the Collaborative Islet Transplant Registry. *Transplantation* 2008;86:1783–1788
- Hering BJ, Kandaswamy R, Ansite JD, et al. Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. *JAMA* 2005;293:830–835
- Bellin MD, Kandaswamy R, Parkey J, et al. Prolonged insulin independence after islet allotransplants in recipients with type 1 diabetes. *Am J Transplant* 2008;8:2463–2470
- CITR Research Group. 2007 update on allogeneic islet transplantation from the Collaborative Islet Transplant Registry (CITR). *Cell Transplant* 2009;18:753–767
- Barton FB, Rickels MR, Alejandro R, et al. Improvement in outcomes of clinical islet transplantation: 1999–2010. *Diabetes Care* 2012;35:1436–1445
- Rickels MR, Liu C, Shlansky-Goldberg RD, et al. Improvement in  $\beta$ -cell secretory capacity after human islet transplantation according to the CIT07 protocol. *Diabetes* 2013;62:2890–2897
- Paty BW, Ryan EA, Shapiro AM, Lakey JR, Robertson RP. Intrahepatic islet transplantation in type 1 diabetic patients does not restore hypoglycemic hormonal counterregulation or symptom recognition after insulin independence. *Diabetes* 2002;51:3428–3434
- Pyzdrowski KL, Kendall DM, Halter JB, Nakhleh RE, Sutherland DE, Robertson RP. Preserved insulin secretion and insulin independence in recipients of islet autografts. *N Engl J Med* 1992;327:220–226
- Kendall DM, Teuscher AU, Robertson RP. Defective glucagon secretion during sustained hypoglycemia following successful islet allo- and auto-transplantation in humans. *Diabetes* 1997;46:23–27
- Gupta V, Wahoff DC, Rooney DP, et al. The defective glucagon response from transplanted intrahepatic pancreatic islets during hypoglycemia is transplantation site-determined. *Diabetes* 1997;46:28–33
- Zhou H, Zhang T, Bogdani M, et al. Intrahepatic glucose flux as a mechanism for defective intrahepatic islet  $\alpha$ -cell response to hypoglycemia. *Diabetes* 2008;57:1567–1574
- Rickels MR, Schutta MH, Mueller R, et al. Islet cell hormonal responses to hypoglycemia after human islet transplantation for type 1 diabetes. *Diabetes* 2005;54:3205–3211
- Rickels MR, Fuller C, Dalton-Bakes C, et al. Restoration of glucose counterregulation by islet transplantation in long-standing type 1 diabetes. *Diabetes* 2015;64:1713–1718
- Bellin MD, Parazzoli S, Oseid E, et al. Defective glucagon secretion during hypoglycemia after intrahepatic but not nonhepatic islet autotransplantation. *Am J Transplant* 2014;14:1880–1886