Perelman School of Medicine
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Adrenergic Contribution to Glucose Counterregulation in Islet Transplantation

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Study Products

Phentolamine (Regitine®)
Propranolol

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Study Summary

Title	Adrenergic Contribution to Glucose Counterregulation in Islet Transplantation
Short Title	Adrenergic System in Islet Transplantation
IRB Number	Pending
Methodology	Within subject and across group mechanistic design.
Study Duration	4.5 years
Study Center	Hospital of the University of Pennsylvania
Objectives	Primary: • To determine the effect of sympathetic neural input on islet cell hormonal responses to insulin-induced hypoglycemia in type 1 diabetic recipients of intrahepatic islet transplantation. • To determine the effect of sympathetic hormonal (epinephrine) input on islet cell hormonal responses to insulin-induced hypoglycemia in type 1 diabetic recipients of intrahepatic islet transplantation. Secondary: • To determine the effect of islet transplant site on islet cell hormonal responses to insulin-induced hypoglycemia in type 1 diabetic recipients of extrahepatic islet transplantation. • To compare islet cell hormonal responses to insulin-induced hypoglycemia between pancreatectomized recipients of intrahepatic islet auto-transplantation and type 1 diabetic recipients of intrahepatic islet transplantation.
Number of Subjects	Up to 15 type 1 diabetic recipients of intrahepatic islet transplantation (Group 1). Up to 15 type 1 diabetic recipients of extrahepatic islet transplantation (Group 2). Up to 15 pancreatectomized recipients of intrahepatic islet auto-transplantation (Group 3).

Main Inclusion and Exclusion Criteria	Group 1 subjects will include adult (ages 21 - 65 years) type 1 diabetic recipients of intrahepatic islet transplants with stable islet graft function defined by C-peptide > 0.5 ng/ml and insulin-independent or insulin-dependent with daily insulin requirement < 0.2 units/kg·d to maintain HbA_{1c} < 7.0%. Subjects will be on standard immunosuppression consisting of tacrolimus with or without sirolimus or mychophenolic acid, and if receiving prednisone, no more than 5 mg daily. Subjects will be excluded for uncontrolled hypertension, use of β-blockers, abnormal kidney, liver, or thyroid function, anemia, seizure disorder not related to prior severe hypoglycemia, pregnancy, and nursing. Subjects with a history of cardiovascular disease will not receive the phentolamine condition and subjects with a history of bronchial asthma will not receive the propranolol condition. Group 2 subjects will include adult (ages 21 - 65 years) type 1 diabetic recipients of extrahepatic islet transplants with stable islet graft function defined by C-peptide > 0.5 ng/ml and insulin-independent or insulin-dependent with daily insulin requirement < 0.2 units/kg·d to maintain HbA_{1c} < 7.0%. Subjects will be on standard immunosuppression consisting of tacrolimus with or without sirolimus or mychophenolic acid, and if receiving prednisone, no more than 5 mg daily. Subjects will be excluded for uncontrolled hypertension, active cardiovascular disease, abnormal kidney, liver, or thyroid function, anemia, seizure disorder not related to prior severe hypoglycemia, pregnancy, and nursing. Group 3 subjects will include adult (ages 21 - 65 years) pancreatectomized recipients of intrahepatic islet auto-transplants with stable islet graft function defined by C-peptide > 0.5 ng/ml and insulin-independent or insulin-dependent with daily insulin requirement < 0.2 units/kg·d to maintain HbA_{1c} < 7.0%. Subjects will be excluded for uncontrolled hypertension, active cardiovascular disease, abnormal kidney, liver, or thyroid function, anemia, seizure disorder, pregnancy, and nursing.
Intervention	Islet cell hormonal responses to a hyperinsulinemic euglycemic-hypoglycemic clamp will be assessed in Group 1 on up to three occasions with randomized, double-blind administration of the α-adrenergic blocker phentolamine, and/or the β-adrenergic blocker propranolol, and placebo. Responses in Group 1 under the placebo condition will be used for comparison to those obtained from hyperinsulinemic euglycemic-hypoglycemic clamp testing on one occasion in subjects in each of Group 2 and Group 3.
Statistical Methodology	Islet cell hormonal responses during α-adrenergic blockage with phentolamine (β-cell C-peptide suppression) and β-adrenergic blockage with propranolol (α-cell glucagon activation) in Group 1 will each be compared against placebo using paired *t* tests or Wilcoxon matched-pairs tests as appropriate. Islet cell hormonal responses (β-cell C-peptide suppression and α-cell glucagon activation) in Group 2 will each be compared against Group 1 responses derived under the placebo condition using unpaired *t* tests or Mann Whitney U tests as appropriate. Islet cell hormonal responses (β-cell C-peptide suppression and α-cell glucagon activation) in Group 3 will each be compared against Group 1 responses derived under the placebo condition using unpaired *t* tests or Mann Whitney U tests as appropriate. Significance will be considered at $P \leq 0.05$ (two-tailed). For additional measures, multiple comparisons are being made without adjustment for multiplicity, and so any findings will be hypothesis generating.
Data and Safety Monitoring Plan	Data quality management and the ongoing safety of subjects will be monitored by the PI and an independent medical monitor who will serve as chair of an internal data and safety monitoring board (DSMB).

Background and Study Rationale

This document is a research protocol and the described study will be conducted in compliance with the provisions set forth in the protocol as well as, Good Clinical Practice standards, associated federal regulations, and all applicable University research requirements. This study will be conducted in full accordance with all applicable University of Pennsylvania Research Policies and Procedures and all applicable Federal and state laws and regulations. All episodes of noncompliance will be documented.

1 Introduction

1.1 Background and Relevant Literature

Hypoglycemia is a major barrier to the achievement of adequate glycemic control for most patients with insulin-dependent diabetes, both those with type 1 diabetes and advanced type 2 diabetes (Cryer, 2008). Inadequate glycemic control can lead to the development of diabetic retinopathy, nephropathy, and neuropathy, the leading causes of adult blindness, kidney failure, and non-traumatic amputation in the United States. The American Diabetes Association treatment guidelines recommend that adults with type 1 diabetes target HbA_{1c} levels < 7.0% unless there is a reason, such as significant hypoglycemia or hypoglycemia unawareness, to set a higher target (2015). However, even with HbA_{1c} < 7.0% the residual risk for cardiovascular and all-cause mortality in patients with type 1 diabetes remains more than twice that in nondiabetics (Lind et al., 2014), with the lowest mortality rates seen with HbA_{1c} ≤ 6.5% (Stadler et al., 2014). Unfortunately, despite tremendous advances in the technology available for insulin delivery and glucose monitoring, only 30% of adults with type 1 diabetes in the United States receiving care at specialty diabetes clinics are achieving a HbA_{1c} level < 7.0% (Miller et al., 2015). Despite this low proportion of patients reaching target average glycemic control, 8% reported experiencing a severe hypoglycemic event resulting in seizure or loss-of-consciousness in the prior 3 months, including 6% of those with a HbA_{1c} level < 7.0%, 8% of those with a HbA_{1c} between 7.0 – 9.0%, and 12% of those with a HbA_{1c} > 9.0% (Miller et al., 2015). Thus, current recommendations to set a higher HbA_{1c} target for patients with significant hypoglycemia or hypoglycemia unawareness (2015) are unlikely to impact the burden of severe hypoglycemia in type 1 diabetes, and may not be acceptable to patients striving to avoid or mitigate the micro- and macrovascular complications of this disease.

Severe episodes of hypoglycemia are life-threatening, fear of such episodes distressing, and the cumulative effects of recurrent hypoglycemia impair neurocognitive function. Diabetes-related death is the most frequent cause of mortality among patients under 30 years-of-age (Skrivarhaug et al., 2006; Feltbower et al., 2008), and while severe hypoglycemia is documented in only ~ 12% of diabetes-related deaths, this is likely an under representation due to the presence of twice as many unexplained diabetes-related deaths. Not uncommonly, young people with type 1 diabetes are found “dead-in-bed” (Tanenberg et al., 2010; Tattersall and Gill, 1991), an unfortunate consequence of likely severe hypoglycemia inducing brain death (Auer, 2004) or a fatal cardiac arrhythmia (Tu et al., 2010). In fact, patients reporting an episode of severe hypoglycemia experience a 3.4-fold increase in mortality over the subsequent 5 years (Mccoy et al., 2012). The risk of experiencing a severe hypoglycemic episode increases with long standing disease due to the progressive development of compromised physiologic defense mechanisms against a falling blood glucose concentration in the setting of therapeutic hyperinsulinemia. By 15 years of disease duration most patients have developed near total loss of functioning β-cells (C-peptide negative) (Tsai et al., 2006) resulting in loss of inhibition of endogenous insulin secretion as well as activation of glucagon secretion in response to declining blood glucose (Cooperberg and Cryer, 2010), which together normally increase endogenous (primarily hepatic) glucose production to circumvent the development of hypoglycemia. In the absence of these islet cell responses to hypoglycemia, epinephrine secretion and autonomic symptom generation become critical to increase endogenous glucose production and alert the individual to ingest food (Cryer et al., 2003). Unfortunately, these sympathoadrenal responses are impaired by recurrent episodes of hypoglycemia leading to a syndrome of hypoglycemia unawareness, also known as hypoglycemia-associated autonomic failure (HAAF) (Cryer, 2013). Hypoglycemia unawareness is associated with a 20-fold increased risk for experiencing life-threatening hypoglycemia (Pedersen-Bjergaard et al., 2004). Clearly, new strategies are required to restore physiologic defense mechanisms against the development of severe hypoglycemia.

Cellular therapy strategies hold particular promise for the alleviation of hypoglycemia in type 1 diabetes. A successful license-enabling phase 3 multi-center clinical trial of islet transplantation sponsored by the National Institutes of Health has been completed, with remarkable improvement over prior approaches in engrafted and surviving functional islet β-cell mass documented in the 11 subjects participating in the trial at Penn (Rickels et al., 2013). These subjects were studied in the first cycle of the grant supporting the present study, which showed that intrahepatic islet transplantation abrogates clinical hypoglycemia (while lowering HbA_{1c} to ≤ 6.5%) with abolition of time spent in the hypoglycemic range (< 60 mg/dl), and results in appropriate inhibition of endogenous insulin secretion (C-peptide suppression) and activation of glucagon secretion with normalization of the endogenous glucose production response during insulin-induced hypoglycemia (Rickels et al., 2015). Furthermore, these subjects experienced normalization of epinephrine secretion and hypoglycemia symptom recognition by 18 months post-transplant (Rickels et al., 2016). This remarkable effect of achieving both ideal average glycemic control and amelioration from hypoglycemia justifies incorporation of islet transplantation in to the treatment algorithm for type 1 diabetes complicated by problematic hypoglycemia (Choudhary et al., 2015), and provides a benchmark to which the development of alternative sources of islet tissue and alternative sites of transplantation should be compared.

The current requirements for deceased donor pancreases to supply islet tissue and for immunosuppression to prevent alloimmune rejection and recurrent autoimmunity preclude broad application of islet transplantation in type 1 diabetes. Thus, pre-clinical and early phase clinical work is focused on creating human stem cell derived β-cells as a source of islet tissue (Rezania et al., 2014; Pagliuca et al., 2014), and immune-isolating devises as vehicles for cellular transplantation (Bruin et al., 2013). The successful translation of these emerging strategies requires a more complete understanding of the mechanisms underlying successful human islet transplantation. The restored activation of glucagon secretion presumably originates from the transplanted islets, where an intraislet decrement in insulin secretion, evidenced by the restored suppression of endogenous insulin secretion (Rickels et al., 2015), provides the paracrine signal for activation of glucagon secretion during hypoglycemia (Cooperberg and Cryer, 2010). Importantly, our work has also demonstrated partial suppression of endogenous insulin secretion during hyperinsulinemic euglycemic clamps that is identical to normal (Rickels et al., 2015). This is significant as insulin is thought to mediate suppression of its own secretion through sympathetic innervation of the islet (Luzi et al., 1992; Boden et al., 1993), and re-innervation of intrahepatic islets by the sympathetic nervous system has been demonstrated in rodents (Gardemann et al., 1994). The present study will assess the contribution of sympathetic neural input to intrahepatically transplanted islets by measuring islet cell hormonal responses during hyperinsulinemia with euglycemia followed by hypoglycemia in islet transplant recipients in the presence and absence of α-adrenergic blockade (**Figure 1**). Mitigation of C-peptide suppression during euglycemia and glucagon activation during hypoglycemia by α-adrenergic blockade would evidence sympathetic neural regulation of the transplanted islet cell responses to hypoglycemia.

Epinephrine independent of hypoglycemia can stimulate glucagon secretion in humans (Gerich et al., 1976), raising the question of whether the improved epinephrine response to insulin-induced hypoglycemia in islet transplant recipients may be stimulating glucagon secretion from native or transplanted α-cells (Robertson, 2015). Against a native α-cell response is evidence that non-transplant patients with long standing type 1 diabetes who have normal epinephrine responses to insulin-induced hypoglycemia still fail to release glucagon (White et al., 1985). Nevertheless, individuals who have undergone pancreatectomy with intrahepatic islet auto-transplantation, and so have no orthotopic α-cells, have been reported to have defective glucagon responses to insulin-induced hypoglycemia, questioning of the ability of liver to supported transplanted α-cell function (Bellin et al., 2014). It has been hypothesized that inhibition of intrahepatic α-cells may occur by locally increased glucose levels (Zhou et al., 2008); however, importantly our work in humans has demonstrated normal and complete suppression of C-peptide during hypoglycemia in intrahepatic islet recipients indicating that the intrahepatic islet β-cells do at least appropriately sense and respond to the degree of peripheral hypoglycemia. The present study will assess the contribution of hormonal epinephrine to glucagon secretion in islet transplant recipients by measuring islet cell hormonal responses to insulin-induced hypoglycemia in the presence and absence of β-adrenergic blockade (**Figure 1**). Lack of an effect of β-adrenergic blockade on

glucagon secretion will confirm that intrahepatically transplanted α-cells, like their neighboring β-cells, do sense and respond to hypoglycemia in type 1 diabetic islet transplant recipients.

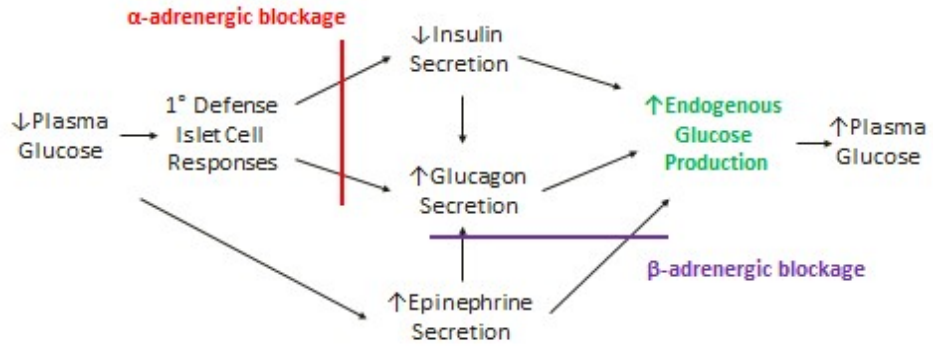

Figure 1. The primary objective of this study is to determine the contribution of sympathetic neural and hormonal (epinephrine) inputs to the islet cell hormonal responses to hypoglycemia in type 1 diabetic islet transplant recipients. Glucose counterregulation during hyperinsulinemic euglycemic-hypoglycemic clamps will be assessed on three randomized, blinded occasions during administration of the α-adrenergic blocker phentolamine, the β-adrenergic blocker propranolol, or placebo.

In patients who have undergone pancreatectomy, and, because of large isolated islet tissue volume, have received auto-transplantation of islets both in the liver and in the peritoneal cavity, a fully normal glucagon response to insulin-induced hypoglycemia has been reported (Bellin et al., 2014). It is not known whether the normal levels of measured glucagon is a consequence of the larger transplanted islet mass, lack of first-pass hepatic extraction of glucagon released from peritoneal islets in to the systemic circulation, or more appropriate exposure of intraperitoneal than intrahepatic islets to systemic glucose levels. As a secondary objective, this study will examine islet cell hormonal responses to insulin-induced hypoglycemia in type 1 diabetic recipients of extrahepatic islet transplantation, and compare the results to those obtained using the same hyperinsulinemic euglycemic-hypoglycemic clamp during placebo infusion in the patients with intrahepatic islets. These results will provide important mechanistic insights in to the potential differences in restoration of glucose counterregulatory defenses in type 1 diabetes by site of islet engraftment.

Moreover, in order to understand the mechanisms underlying the potential differences in the glucagon response to insulin-induced hypoglycemia between pancreatectomized individuals who have received intrahepatic islet auto-transplantation and type 1 diabetic recipients of intrahepatic islet transplantation, this study will assess islet cell hormonal responses to insulin-induced hypoglycemia in both groups of patients. Results obtained from pancreatectomized recipients of islet auto-transplantation will be compared to those obtained using the same hyperinsulinemic euglycemic-hypoglycemic clamp during placebo infusion in the patients with intrahepatic islets. We hypothesize finding differences in additional counterregulatory mechanisms such as the epinephrine response, which while restored in type 1 diabetic recipients of intrahepatic islet transplantation may be impaired together with the glucagon response following pancreatectomy that requires roux-en-Y gastrojejunal reconstruction associated with late dumping alimentary hypoglycemia that could induce HAAF (Abrahamsson et al., 2016).

2 Study Objectives

2.1 Primary Objectives

- To determine the effect of sympathetic neural input on islet cell hormonal responses to insulin-induced hypoglycemia in type 1 diabetic recipients of intrahepatic islet transplantation.

- To determine the effect of sympathetic hormonal (epinephrine) input on islet cell hormonal responses to insulin-induced hypoglycemia in type 1 diabetic recipients of intrahepatic islet transplantation.

This study is designed to test the hypothesis that α-adrenergic (neural) blockade will abolish insulin-mediated suppression of C-peptide, attenuating α-cell glucagon secretion during hypoglycemia, and that β-adrenergic (hormonal) blockade will have no effect. Glucose counterregulatory responses will be measured during hyperinsulinemic euglycemic-hypoglycemic clamps on three occasions with randomized, double-blind administration of the α-adrenergic blocker phentolamine, the β-adrenergic blocker propranolol, or placebo. The demonstration of neural rather than hormonal regulation of the transplanted islet cell response to hypoglycemia is critical for understanding the mechanism for protection from hypoglycemia afforded by intrahepatically transplanted islets.

2.2 Secondary Objectives

- To determine the effect of islet transplant site on islet cell hormonal responses to insulin-induced hypoglycemia in type 1 diabetic recipients of extrahepatic islet transplantation.

Glucose counterregulation has not been studied in type 1 diabetic recipients of extrahepatic islet transplantation. Comparison of glucose counterregulatory responses measured during hyperinsulinemic euglycemic-hypoglycemic clamps will be compared to those obtained from type 1 diabetic recipients of intrahepatic islet transplantation studied under the placebo condition above.

- To compare islet cell hormonal responses to insulin-induced hypoglycemia between pancreatectomized recipients of intrahepatic islet auto-transplantation and type 1 diabetic recipients of intrahepatic islet transplantation.

Glucose counterregulation has not been directly compared between recipients of intrahepatic auto- and allo-islet transplantation. Direct comparison of glucose counterregulatory responses under the same experimental conditions is required to understand whether mechanisms other than the glucagon response may be important to the reported hypoglycemia affecting pancreatectomized recipients of islet auto-transplantation (Lin et al., 2016).

3 Investigational Plan

3.1 Design

Group 1 subjects will include adult (ages 21 - 65 years) type 1 diabetic recipients of intrahepatic islet transplants with stable islet graft function defined by C-peptide > 0.5 ng/ml and insulin-independent or insulin-dependent with daily insulin requirement < 0.2 units/kg·d to maintain HbA_{1c} < 7.0%. Subjects will be on standard immunosuppression consisting of tacrolimus with or without sirolimus or mychophenolic acid, and if receiving prednisone, no more than 5 mg daily. Subjects will be excluded for uncontrolled hypertension, use of β-blockers, abnormal kidney, liver, or thyroid function, anemia, seizure disorder not related to prior severe hypoglycemia, pregnancy, and nursing. Subjects with a history of cardiovascular disease will not receive the phentolamine condition and subjects with a history of bronchial asthma will not receive the propranolol condition. After informed consent, eligibility will be determined by a screening visit to include medical history, physical exam, EKG, urine pregnancy test, serum chemistries, TSH, cell counts, HbA_{1c} and C-peptide. Detailed inclusion/exclusion criteria are provided below.

Eligible Group 1 subjects will undergo placement of a 7 day blinded continuous glucose monitor (CGM; iPro®2 Professional Continuous Glucose Monitor, Medtronic, Northridge, CA) to characterize their hypoglycemia exposure, and be randomized to the order of receiving the α-adrenergic blocker phentolamine, the β-adrenergic blocker propranolol, or placebo in a double-blind cross-over fashion during each of three study visits. Subjects will avoid strenuous activity for three days prior to each study visit, with each visit occurring at least one week but not longer than one month apart. Subjects will be invited to spend the night in the Penn Center for Human Phenomic Science the evening prior to each study day, and will be required to do so if using insulin to maintain near-normoglycemia. Morning

medications will be held until the completion of testing, with the exception of morning doses of twice daily immunosuppressants given after baseline blood samples are collected. Following 10-hours of overnight fasting, around 0700 at *t* = -120 min a primed (5 mg/kg · fasting plasma glucose/90 for 5 min) continuous (0.05 mg/kg·min for 355 min) infusion of 6,6-$^{2}H_{2}$-glucose (99% enriched; Cambridge Isotopes Laboratories, Andover, MA) will be administered to assess endogenous glucose production before and during the induction of hyperinsulinemia (Bernroider et al., 2005; Rickels et al., 2015). The phentolamine (0.95 µg/kg·min), propranolol (0.48 µg/kg·min), and placebo will each be administered intravenously, with the infusion starting at *t* = -30 min together with initiation of cardiac telemetry monitoring. These doses of phentolamine and propranolol have been shown to be effective in blocking blood pressure increases induced by phenylephrine (an α_1-receptor agonist), and heart rate increases induced by isoproterenol (a β_1-receptor agonist), respectively (Girdler et al., 1993). Heart rate and blood pressure will be recorded at least every fifteen minutes for protocol driven reduction, cessation, or reintroduction of the phentolamine, propranolol, or placebo infusion. After baseline blood samples at t = -15, and -1 min, at t = 0 min a continuous infusion of insulin (1.0 mU/kg·min for 180 min) will be administered to produce hyperinsulinemia. Subsequently, a variable rate infusion of 20% glucose will be initiated to target the plasma glucose at ~ 90 mg/dl until 90 min (euglycemic phase), and then ~ 50 mg/dl until 180 min (hypoglycemic phase). To reduce changes in plasma enrichment during the clamp, the variable glucose infusion will be enriched to ~ 2.0 % with 6,6-$^{2}H_{2}$-glucose (Bernroider et al., 2005; Rickels et al., 2015). Blood samples will be taken every 5 min, centrifuged, and measured at bedside with an automated glucose analyzer (YSI 2300; Yellow Springs Instruments, Yellow Springs, OH) to adjust the glucose infusion rate and achieve the desired plasma glucose concentration. Additional blood samples will be taken at t = 30, 60, 75, 90, 120, 150, 165, and 180 min for biochemical analysis and verification of the plasma glucose levels. All samples will be collected on ice in chilled tubes containing EDTA and Protease Inhibitor Cocktail (Sigma-Aldrich, St. Louis, MO), centrifuged at 4°C, separated, and frozen at -80°C for subsequent analysis. The total amount of blood sampled will be 100 ml. A questionnaire will be administered every 15 - 30 min during the study in order to quantitate autonomic symptoms as the sum of scores ranging from 0 (none) to 5 (severe) for each of the following symptoms: anxiety, palpitations, sweating, tremor, hunger, and tingling (Towler et al., 1993; Rickels et al., 2015).

To ensure complete α-blockade and complete β-blockade from the phentolamine and propranolol infusions, respectively, cardiovascular (heart rate and blood pressure) responses during the three testing conditions will be assessed after the first 3 subjects complete study. If no reduction in BP or increase in heart rate is evident during phentolamine administration, the infusion rate will be doubled for the next 3 subjects. If no reduction in heart rate or blood pressure is observed during propranolol administration, the infusion rate will be doubled for the next 3 subjects. Cardiovascular responses will again be assessed, and consideration made to reducing, but not further increasing, the rate of phentolamine or propranolol infusion. These assessments will be made by the PI together with the medical monitor, Dr. Raymond Townsend, Professor of Medicine in the Nephrology & Hypertension Division, who will also serve as DSMB chair. Conditions of testing will remain double-blind for each subject until their completion of all testing visits, unless for safety concerns, either the PI or medical monitor request an unblinding.

Group 2 subjects will include adult (ages 21 - 65 years) type 1 diabetic recipients of extrahepatic islet transplants with stable islet graft function defined by C-peptide > 0.5 ng/ml and insulin-independent or insulin-dependent with daily insulin requirement < 0.2 units/kg·d to maintain HbA_{1c} < 7.0%. Subjects will be on standard immunosuppression consisting of tacrolimus with or without sirolimus or mychophenolic acid, and if receiving prednisone, no more than 5 mg daily. Subjects will be excluded for uncontrolled hypertension, active cardiovascular disease, abnormal kidney, liver, or thyroid function, anemia, seizure disorder not related to prior severe hypoglycemia, pregnancy, and nursing. After informed consent, eligibility will be determined by a screening visit to include medical history, physical exam, EKG, urine pregnancy test, serum chemistries, TSH, cell counts, HbA_{1c} and C-peptide. Detailed inclusion/exclusion criteria are provided below.

Eligible Group 2 subjects will undergo placement of a 7 day blinded CGM (iPro®2 Professional) to characterize their hypoglycemia exposure, and participate in one study visit. Subjects will avoid strenuous activity for three days prior to the study visit. Subjects will be invited to spend the night in the Penn Center for Human Phenomic Science the evening prior to the study visit day, and will be required to do so if using insulin to maintain near-normoglycemia. Morning medications will be held until the

completion of testing, with the exception of morning doses of twice daily immunosuppressants given after baseline blood samples are collected. Following 10-hours of overnight fasting, around 0700 at *t* = -120 min a primed (5 mg/kg · fasting plasma glucose/90 for 5 min) continuous (0.05 mg/kg·min for 355 min) infusion of 6,6-$^{2}H_{2}$-glucose (99% enriched; Cambridge Isotopes Laboratories) will be administered to assess endogenous glucose production before and during the induction of hyperinsulinemia (Bernroider et al., 2005; Rickels et al., 2015). At *t* = -30 min cardiac telemetry monitoring will be initiated, with every 30 min monitoring of heart rate and blood pressure documented during the glucose clamp procedure. After baseline blood samples at t = -15, and -1 min, at t = 0 min a continuous infusion of insulin (1.0 mU/kg·min for 180 min) will be administered to produce hyperinsulinemia. Subsequently, a variable rate infusion of 20% glucose will be initiated to target the plasma glucose at ~ 90 mg/dl until 90 min (euglycemic phase), and then ~ 50 mg/dl until 180 min (hypoglycemic phase). To reduce changes in plasma enrichment during the clamp, the variable glucose infusion will be enriched to ~ 2.0 % with 6,6-$^{2}H_{2}$-glucose (Bernroider et al., 2005; Rickels et al., 2015). Blood samples will be taken every 5 min, centrifuged, and measured at bedside with an automated glucose analyzer (YSI 2300) to adjust the glucose infusion rate and achieve the desired plasma glucose concentration. Additional blood samples will be taken at t = 30, 60, 75, 90, 120, 150, 165, and 180 min for biochemical analysis and verification of the plasma glucose levels. All samples will be collected on ice in chilled tubes containing EDTA and Protease Inhibitor Cocktail, centrifuged at 4°C, separated, and frozen at -80°C for subsequent analysis. The total amount of blood sampled will be 100 ml. A questionnaire will be administered every 15 - 30 min during the study in order to quantitate autonomic symptoms as the sum of scores ranging from 0 (none) to 5 (severe) for each of the following symptoms: anxiety, palpitations, sweating, tremor, hunger, and tingling (Towler et al., 1993; Rickels et al., 2015).

Group 3 subjects will include adult (ages 21 - 65 years) pancreatectomized recipients of intrahepatic islet auto-transplants with stable islet graft function defined by C-peptide > 0.5 ng/ml and insulin-independent or insulin-dependent with daily insulin requirement < 0.2 units/kg·d to maintain HbA_{1c} < 7.0%. Subjects will be excluded for uncontrolled hypertension, active cardiovascular disease, abnormal kidney, liver, or thyroid function, anemia, seizure disorder, pregnancy, and nursing. After informed consent, eligibility will be determined by a screening visit to include medical history, physical exam, EKG, urine pregnancy test, serum chemistries, TSH, cell counts, HbA_{1c} and C-peptide. Detailed inclusion/exclusion criteria are provided below.

Eligible Group 3 subjects will undergo placement of a 7 day blinded CGM (iPro®2 Professional) to characterize their hypoglycemia exposure, and participate in one study visit. Subjects will avoid strenuous activity for three days prior to the study visit. Subjects will be invited to spend the night in the Penn Center for Human Phenomic Science the evening prior to the study visit day, and will be required to do so if using insulin to maintain near-normoglycemia. Morning medications will be held until the completion of testing. Following 10-hours of overnight fasting, around 0700 at *t* = -120 min a primed (5 mg/kg · fasting plasma glucose/90 for 5 min) continuous (0.05 mg/kg·min for 355 min) infusion of 6,6-$^{2}H_{2}$-glucose (99% enriched; Cambridge Isotopes Laboratories) will be administered to assess endogenous glucose production before and during the induction of hyperinsulinemia (Bernroider et al., 2005; Rickels et al., 2015). At *t* = -30 min cardiac telemetry monitoring will be initiated, with every 30 min monitoring of heart rate and blood pressure documented during the glucose clamp procedure. After baseline blood samples at t = -15, and -1 min, at t = 0 min a continuous infusion of insulin (1.0 mU/kg·min for 180 min) will be administered to produce hyperinsulinemia. Subsequently, a variable rate infusion of 20% glucose will be initiated to target the plasma glucose at ~ 90 mg/dl until 90 min (euglycemic phase), and then ~ 50 mg/dl until 180 min (hypoglycemic phase). To reduce changes in plasma enrichment during the clamp, the variable glucose infusion will be enriched to ~ 2.0 % with 6,6-$^{2}H_{2}$-glucose (Bernroider et al., 2005; Rickels et al., 2015). Blood samples will be taken every 5 min, centrifuged, and measured at bedside with an automated glucose analyzer (YSI 2300) to adjust the glucose infusion rate and achieve the desired plasma glucose concentration. Additional blood samples will be taken at t = 30, 60, 75, 90, 120, 150, 165, and 180 min for biochemical analysis and verification of the plasma glucose levels. All samples will be collected on ice in chilled tubes containing EDTA and Protease Inhibitor Cocktail, centrifuged at 4°C, separated, and frozen at -80°C for subsequent analysis. The total amount of blood sampled will be 100 ml. A questionnaire will be administered every 15 - 30 min during the study in order to quantitate autonomic symptoms as the sum of scores ranging from 0 (none) to 5 (severe) for each of the following

symptoms: anxiety, palpitations, sweating, tremor, hunger, and tingling (Towler et al., 1993; Rickels et al., 2015).

Biochemical Analysis: Plasma glucose and lactate will be determined in duplicate by the glucose oxidase method using an automated glucose analyzer (YSI 2300). Plasma insulin, C-peptide, and glucagon will be measured in duplicate by double-antibody radioimmunoassays (Millipore, Billerica, MA) at the Penn Diabetes Research Center. Plasma epinephrine and norepinephrine will be measured by high-performance liquid chromatography with electrochemical detection at the Penn Clinical Center for Epidemiology & Biostatistics Laboratory. Enrichment of 6,6-$^{2}H_{2}$-glucose will be measured by gas chromatography-mass spectrometry analysis by the Metabolic Tracer Resource of the Penn Institute for Diabetes, Obesity & Metabolism. Samples from all three experiments for a given subject in Group 1 will be assayed simultaneously, and when possible, together with samples from a representative subject from Group 2 and/or Group 3.

Calculations: We will define inhibitory responses during the euglycemic phase as the levels of C-peptide and glucagon between 60 – 90 min. We will define counterregulatory responses during the hypoglycemic phase as the levels of C-peptide, glucagon, epinephrine, autonomic symptoms, and endogenous glucose production between 150 – 180 min. If for some reason baseline levels at each study visit vary, we will analyze the incremental responses from baseline, or adjust for baseline data in the statistical analysis. The rate of appearance (R_a) of glucose during the clamps will be calculated using Steele's non-steady state equation modified for the use of stable isotopes as previously described (Wolfe and Chinkes, 2005; Rickels et al., 2014). Endogenous glucose production during the clamp will be calculated from the difference between R_a and the exogenous glucose infusion rate.

3.2 Study Endpoints

3.2.1 Primary Study Endpoint

The primary outcome measures will be the levels of C-peptide suppression during hyperinsulinemia euglycemia (between 60 – 90 min) and of glucagon activation during hyperinsulinemia hypoglycemia (between 150 – 180 min).

3.2.2 Secondary Study Endpoints

Secondary outcome measures will include levels of epinephrine, autonomic symptoms, and rates of endogenous glucose production during hyperinsulinemia hypoglycemia (between 150 – 180 min).

4 Study Population and Duration of Participation

4.1 Duration of Study Participation

Group 1 subjects may participate for up to 3 months in order to complete their screening visit and 2 or 3 study visits, each at least one week and not more than one month apart. Group 2 and 3 subjects will complete their study visit within one month of their screening visit.

4.2 Total Number of Subjects and Sites

We plan on enrolling up to 15 subjects in Group 1 to obtain 12 completers for the phentolamine vs. placebo and propranolol vs. placebo comparisons. We plan on enrolling up to 15 subjects in each of Groups 2 and 3 to obtain 12 completers in each group for comparison vs. Group 1 (placebo). All visits will occur at Penn; however, recruitment will involve referral of islet transplant recipients from collaborating institutions (letters of support are available in the grant).

4.3 Inclusion Criteria

Group 1 will involve subjects with long standing type 1 diabetes who underwent intrahepatic islet transplantation and have on-going stable insulin-independent or partially insulin-dependent islet graft function. The inclusion criteria are:

Patients who meet *all* of the following criteria are eligible for participation in **Group 1** of this study:

1. Male and female subjects age 21 to 65 years of age.
2. Subjects who are able to provide written informed consent and to comply with the procedures of the study protocol.
3. Clinical history compatible with type 1 diabetes with onset of disease at < 40 years of age and insulin-dependent for > 10 years at the time of islet transplantation > 6 months before study.
4. Stable islet graft function defined by C-peptide > 0.5 ng/ml and insulin-independent or insulin-dependent with daily insulin requirement < 0.2 units/kg·d to maintain HbA_{1c} < 7.0%.
5. Use of standard immunosuppression consisting of tacrolimus with or without sirolimus or mychophenolic acid. Substitutions of tacrolimus with cyclosporine, and of sirolimus or mychophenolic acid with azathioprine are permissible if stable for over 3 months. Prednisone is allowable if no more than 5 mg daily.

Group 2 will involve subjects with long standing type 1 diabetes who underwent extrahepatic islet transplantation and have on-going stable insulin-independent or partially insulin-dependent islet graft function. The inclusion criteria are:

Patients who meet *all* of the following criteria are eligible for participation in **Group 2** of this study:

1. Male and female subjects age 21 to 65 years of age.
2. Subjects who are able to provide written informed consent and to comply with the procedures of the study protocol.
3. Clinical history compatible with type 1 diabetes with onset of disease at < 40 years of age and insulin-dependent for > 10 years at the time of islet transplantation > 6 months before study.
4. Stable islet graft function defined by C-peptide > 0.5 ng/ml and insulin-independent or insulin-dependent with daily insulin requirement < 0.2 units/kg·d to maintain HbA_{1c} < 7.0%.
5. Use of standard immunosuppression consisting of tacrolimus with or without sirolimus or mychophenolic acid. Substitutions of tacrolimus with cyclosporine, and of sirolimus or mychophenolic acid with azathioprine are permissible if stable for over 3 months. Prednisone is allowable if no more than 5 mg daily.

Group 3 will involve subjects with pancreatectomy who underwent intrahepatic islet auto-transplantation and have on-going stable insulin-independent or partially insulin-dependent islet graft function. The inclusion criteria are:

Patients who meet *all* of the following criteria are eligible for participation in **Group 3** of this study:

1. Male and female subjects age 21 to 65 years of age.
2. Subjects who are able to provide written informed consent and to comply with the procedures of the study protocol.
3. Clinical history compatible with total pancreatectomy and autologous islet transplantation > 6 months before study.
4. Stable islet graft function defined by C-peptide > 0.5 ng/ml and insulin-independent or insulin-dependent with daily insulin requirement < 0.2 units/kg·d to maintain HbA_{1c} < 7.0%.

4.4 Exclusion Criteria

Patients who meet *any* of the following criteria are *not* eligible for participation in **Group 1** of this study:

1. BMI ≥ 30 kg/m^2.
2. Insulin requirement of ≥ 0.2 units/kg·day.
3. HbA_{1c} ≥ 7.0%.
4. Uncontrolled hypertension: systolic blood pressure > 160 mmHg or diastolic blood pressure > 100 mmHg.
5. History of cardiovascular disease, including coronary artery, cerebrovascular or peripheral vascular disease, or current use of β-blocker therapy. Participants with cardiovascular disease will not participate in the hyperinsulinemic clamp with the phentolamine condition.

6. Bronchial asthma. Participants with bronchial asthma will not participate in the hyperinsulinemic clamp with the propranolol condition.
7. Abnormal kidney function: eGFR < 50 ml/min/1.73 m^2.
8. Abnormal liver function: persistent elevation of liver function tests > 1.5 times the upper limit of normal.
9. Untreated hypothyroidism, Addison's disease, or Celiac disease.
10. Anemia: baseline hemoglobin concentration < 11 g/dl in women and < 12 g/dl in men.
11. Presence of a seizure disorder not related to prior severe hypoglycemia.
12. Use of glucocorticoids greater than 5 mg of prednisone daily, or an equivalent physiologic dose of hydrocortisone.
13. For female participants of child-bearing potential: Positive pregnancy test, presently breast-feeding, or unwillingness to use effective contraceptive measures for the duration of study participation. Oral contraceptives, intra-uterine devices, Norplant®, Depo-Provera®, and barrier devices with spermicide are acceptable contraceptive methods; condoms used alone are not acceptable.
14. Treatment with any anti-diabetic medication other than insulin within 4 weeks of enrollment.
15. Use of any investigational agents within 4 weeks of enrollment.
16. Any medical condition that, in the opinion of the PI, will interfere with the safe completion of the study

Patients who meet *any* of the following criteria are *not* eligible for participation in **Group 2** of this study:

1. BMI ≥ 30 kg/m^2.
2. Insulin requirement of ≥ 0.2 units/kg·day.
3. HbA_{1c} ≥ 7.0%.
4. Uncontrolled hypertension: systolic blood pressure > 160 mmHg or diastolic blood pressure > 100 mmHg.
5. Active cardiovascular disease, including coronary artery, cerebrovascular or peripheral vascular disease.
6. Abnormal kidney function: eGFR < 50 ml/min/1.73 m^2.
7. Abnormal liver function: persistent elevation of liver function tests > 1.5 times the upper limit of normal.
8. Untreated hypothyroidism, Addison's disease, or Celiac disease.
9. Anemia: baseline hemoglobin concentration < 11 g/dl in women and < 12 g/dl in men.
10. Presence of a seizure disorder not related to prior severe hypoglycemia.
11. Use of glucocorticoids greater than 5 mg of prednisone daily, or an equivalent physiologic dose of hydrocortisone.
12. For female participants of child-bearing potential: Positive pregnancy test, presently breast-feeding, or unwillingness to use effective contraceptive measures for the duration of study participation. Oral contraceptives, intra-uterine devices, Norplant®, Depo-Provera®, and barrier devices with spermicide are acceptable contraceptive methods; condoms used alone are not acceptable.
13. Treatment with any anti-diabetic medication other than insulin within 4 weeks of enrollment.
14. Use of any investigational agents within 4 weeks of enrollment.
15. Any medical condition that, in the opinion of the PI, will interfere with the safe completion of the study

Patients who meet *any* of the following criteria are *not* eligible for participation in **Group 3** of this study:

1. BMI ≥ 30 kg/m^2.
2. Insulin requirement of ≥ 0.2 units/kg·day.
3. HbA_{1c} ≥ 7.0%.
4. Uncontrolled hypertension: systolic blood pressure > 160 mmHg or diastolic blood pressure > 100 mmHg.
5. Active cardiovascular disease, including coronary artery, cerebrovascular or peripheral vascular disease.
6. Abnormal kidney function: eGFR < 50 ml/min/1.73 m^2.
7. Abnormal liver function: persistent elevation of liver function tests > 1.5 times the upper limit of normal.

8. Anemia: baseline hemoglobin concentration < 11 g/dl in women and < 12 g/dl in men.
9. Presence of a seizure disorder not related to prior severe hypoglycemia.
10. Use of glucocorticoids greater than 5 mg of prednisone daily, or an equivalent physiologic dose of hydrocortisone.
11. For female participants of child-bearing potential: Positive pregnancy test, presently breast-feeding, or unwillingness to use effective contraceptive measures for the duration of study participation. Oral contraceptives, intra-uterine devices, Norplant®, Depo-Provera®, and barrier devices with spermicide are acceptable contraceptive methods; condoms used alone are not acceptable.
12. Treatment with any anti-diabetic medication other than insulin within 4 weeks of enrollment.
13. Use of any investigational agents within 4 weeks of enrollment.
14. Any medical condition that, in the opinion of the PI, will interfere with the safe completion of the study

4.5 Subject Recruitment

Subjects will be recruited from the PI's clinical practice and from referring physicians' islet transplant practices at collaborating institutions (letters of support are available in the grant). Also, potential subjects may respond to the website for the Penn Institute for Diabetes, Obesity & Metabolism, the study posting on Clinicaltrials.gov, and advertisement through the IRB-approved secure on-line system iConnect. All recruitment materials which will be seen by potential participants will be approved by the Penn IRB.

4.6 Vulnerable Populations:

Children, pregnant women, fetuses, neonates, or prisoners are not included in this research study.

5 Study Procedures

5.1 Screening visit

Once informed consent is obtained as described below, subjects will undergo a history and physical examination, review of their insulin requirements and glucometer download (if applicable), EKG, fasting serum biochemistries (glucose, C-peptide, electrolytes, creatinine, and liver function tests), HbA1c, TSH, complete blood count, and HCG (females). Subjects will have a blinded 7 day CGM (iPro®2 Professional) placed (to be returned by mail) to characterize their hypoglycemia exposure. Subjects who may experience difficulty affording a sufficient supply of test strips for their glucometer will be provided an OneTouch device for use during placement of the CGM in order to maintain proper calibration of the devise. Subjects will complete two hypoglycemia questionnaires. One required to calculate the Clarke score of symptom awareness and the other to quantitate hypoglycemia severity.

5.2 Study visit(s)

5.2.1 Group 1

Eligible Group 1 subjects will be randomized by the Penn Investigational Drug Service to the order of receiving the α-adrenergic blocker phentolamine, and / or the β-adrenergic blocker propranolol, or placebo in a double-blind cross-over fashion during each of three study visits during conduct of a hyperinsulinemic euglycemic-hypoglycemic clamp as described above under **Design**. The Investigational Drug Service will be notified in advance if a subject will not be participating in either the phentolamine or the propranolol condition. Each infusion of phentolamine, propranolol, or placebo will be for 3.5 hours. The dose of phentolamine will be 0.95 μg/kg·min, which will provide a total dose of 0.20 mg/kg that has been shown to block blood pressure increases induced by the α_1-receptor agonist phenylephrine (Girdler et al., 1993), and for a 70 kg subject equates to 4 mg/hour. The dose of propranolol will be 0.48 μg/kg·min, which will provide a total dose of 0.10 mg/kg that has been shown to block heart rate increases induced by the β_1-receptor agonist isoproterenol (Girdler et al., 1993), and for a 70 kg subject equates to 2 mg/hour. All experiments will be conducted with continuous heart rate monitoring using a clinical telemetry unit, and heart rate and automated blood pressure recordings will be made at least every 15 minutes. To minimize the risk for a change in heart rate or blood pressure from either the

phentolamine or propranolol infusion, the blinded infusion will be reduced by 50% and then turned off and then resumed at 50% based on changes in heart rate > 20 bpm and for absolute rates < 50 bpm or > 100 bpm, and for changes in BP of > 20 mmHg or for absolute measures <90/60 mmHg or > 160/100 mmHg. At the end of the hyperinsulinemic euglycemic-hypoglycemic clamp, the phentolamine, propranolol, or placebo infusion as well as the insulin infusion will be turned off, and saline and dextrose infusions will be maintained until stability of BP and heart rate by orthostatic assessment and of glucose by plasma or blood assessment is obtained. During this time the subject will receive lunch in anticipation of discharge two hours after the completion of testing.

5.2.2 Group 2

Eligible Group 2 subjects will undergo one study visit for completion of the hyperinsulinemic euglycemic-hypoglycemic clamp as described above under **Design**. At the end of the clamp test, the insulin infusion will be turned off, and the dextrose infusion will be maintained until stability of glucose by plasma or blood assessment is obtained. During this time the subject will receive lunch in anticipation of discharge one hour after the completion of testing.

5.2.3 Group 3

Eligible Group 3 subjects will undergo one study visit for completion of the hyperinsulinemic euglycemic-hypoglycemic clamp as described above under **Design**. At the end of the clamp test, the insulin infusion will be turned off, and the dextrose infusion will be maintained until stability of glucose by plasma or blood assessment is obtained. During this time the subject will receive lunch in anticipation of discharge one hour after the completion of testing.

5.3 Subject Withdrawal

Subjects may withdraw from the study at any time without impact to their care. They may also be discontinued from the study at the discretion of the PI for lack of adherence to study procedures or visit schedules, AEs, or difficulty completing study procedures, for example due to poor intravenous access. The PI may also withdraw subjects who violate the study plan, to protect the subject for reasons related to safety, or for administrative reasons. It will be documented whether or not each subject completes the study.

6 Statistical Plan

6.1 Sample Size and Power Determination

Based on our published work (Rickels et al., 2016) involving 10 type 1 diabetic recipients of intrahepatic islet transplantation with durable islet graft function at 18 months, the mean ± S.D. C-peptide at baseline (no response) is 0.95 ± 0.29 ng/ml and suppressed during the second hour of hyperinsulinemia euglycemia (response) to 0.54 ± 0.18 ng/ml. To detect a 0.41 ng/ml difference between α-adrenergic blockade with phentolamine and placebo will require 8 subject completers to have > 80% power require at $\alpha = 0.05$ (two-tailed) using a paired t-test. For the same cohort the mean ± S.D. glucagon response during the second hour of hyperinsulinemia euglycemia (no response) is 36 ± 10 pg/ml and when activated during the fourth hour of hyperinsulinemia hypoglycemia (full response) is 61 ± 27 pg/ml. To detect a 25 pg/ml difference between β-adrenergic blockade with propranolol and placebo will require 12 subject completers to have > 80% power at $\alpha = 0.05$ (two-tailed) using a paired t-test. We plan on studying up to 15 subjects in Group 1 to ensure at least 12 completers for the phentolamine vs. placebo and propranolol vs. placebo comparisons; the phentolamine and propranolol visits will not be compared. We also plan on studying up to 15 subjects in each of Groups 2 and 3 to target a similar number of completers in each group that will have similar power for detecting differences in islet cell hormonal responses to insulin-induced hypoglycemia in comparison to Group 1 under the placebo condition.

6.2 Statistical Methods

The counterregulatory responses during α-adrenergic blockade with phentolamine and β-adrenergic blockade with propranolol will each be compared against placebo using paired t-tests or Wilcoxon matched-pairs tests as appropriate, and significance will be considered at $P \leq 0.05$ (two-tailed) for the

primary outcome measures of β-cell C-peptide suppression for phentolamine and α-cell glucagon activation for propranolol. For the additional measures, multiple comparisons are being made without adjustments for multiplicity, and so any findings will be hypothesis generating.

Islet cell hormonal responses (β-cell C-peptide suppression and α-cell glucagon activation) in Group 2 and in Group 3 will each be compared against Group 1 responses derived under the placebo condition using unpaired t-tests or Mann Whitney U tests as appropriate, with significance considered at $P \leq 0.05$ (two-tailed). Secondary outcome measures including epinephrine, autonomic symptoms, and rates of endogenous glucose production during insulin-induced hypoglycemia will be compared similarly without adjustments for multiplicity, and so any finding will be hypothesis generating.

6.3 Control of Bias and Confounding (if applicable, typically observational study or if randomization is not taking place)

Between group comparisons may be adjusted for age, sex, BMI, glycemic control (HbA1c), graft function (C-peptide), or insulin requirements should significant differences in these demographic variables be present.

7 Safety and Adverse Events

7.1 Definitions

7.1.1 Adverse Event

An **adverse event** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

7.1.2 Serious Adverse Event

Serious Adverse Event

Adverse events are classified as serious or non-serious. A **serious adverse event** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- required intervention to prevent permanent impairment or damage
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious will be regarded as **non-serious adverse events**.

7.2 Recording of Adverse Events

At each contact with the subject, an investigator will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results will be recorded in the source document, and will be grouped under one diagnosis.

All adverse events occurring during each subject's participation in the study will be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study intervention or participation is not the cause. Serious adverse events that are still ongoing at the end of study participation will be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study intervention or study participation will be recorded and reported.

7.3 Relationship of AE to Study

The relationship of each adverse event to the study procedures will be characterized by the PI and classified as definitely related, probably related, possibly related, unlikely related, or unrelated.

7.4 Reporting of Adverse Events and Unanticipated Problems

The PI will promptly notify the Penn IRB of all on-site unanticipated, adverse events that are related to the research activity. Other unanticipated problems related to the research involving risk to subjects or others will also be reported promptly. Written reports will be filed using the HS-ERA and in accordance with the Penn IRB timeline of 10 working days.

7.4.1 Follow-up Report

If an AE has not resolved at the time of the initial report and new information arises that changes the investigator's assessment of the event, a follow-up report including all relevant new or reassessed information (e.g., concomitant medication, medical history) will be submitted to the IRB. The PI will be responsible for ensuring that all SAEs are followed until either resolved or stable.

7.4.2 Data and Safety Monitoring Plan

The PI will oversee the safety of the study. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above. In addition, regulatory monitoring will be conducted by a study monitor, and safety monitoring will be conducted by a medical monitor and a data and safety monitoring board (DSMB) as described in the data and safety monitoring plan (DSMP).

7.4.2.1 Data Safety Monitoring Board (if applicable)

A data and safety monitoring board (DSMB) will be established. The mission of the DSMB will be to ensure that risk associated with participation in research is being minimized to the extent practical. This mission will be accomplished by charging the DSMB to determine safe and effective conduct of the study protocol and to recommend modification or conclusion of the protocol when significant benefits or risks have developed or the study is unlikely to be concluded successfully. The DSMB will consist of 3 members independent from the study team, with at least one being a clinical investigator and at least one an endocrinologist experienced in the care of subjects with type 1 diabetes. A CV for each member will be obtained and updated annually. The CVs will be kept on file in the Regulatory Binder to document the qualifications of the DSMB members. One member will serve as chair for meetings of the DSMB, and as the medical monitor available to the PI on an as needed basis to review study design and subject data related to safety concerns. Dr. Raymond Townsend, Professor of Medicine in the Nephrology & Hypertension Division, Associate Director of the Clinical & Translational Research Center, and Member of the Institutional Review Board, will serve as the DSMB chair/medical monitor (letter of support is available in the grant). Dr. Townsend's clinical expertise in hypertension and the use of intravenous phentolamine and propranolol together with his research expertise in the measurement of vascular compliance, glucose clamps, and stable isotopes make him well suited to serve as the DSMB chair for the present study.

The DSMB will meet either by teleconference, or if possible, in person, at least annually and more often if requested due to the appearance of unexpected and possibly related serious adverse events, or an unexpected number of serious adverse events regardless of their relatedness. Each member will receive a monitoring manual including the current protocol, most recent approved consent forms, CRFs, and DSMP. The annual DSMB meeting will be scheduled as soon as possible following submission of the annual reports to the IRB, and those reports containing all adverse events will be reviewed by the DSMB together with any available interim efficacy data. All unexpected and possibly related serious adverse events will be reported expeditiously to both the IRB and the DSMB chair; all other serious adverse events regardless of their relatedness will be reported expeditiously to the DSMB chair. Based on such reporting, the DSMB chair may convene a meeting of the board at any time.

Each DSMB meeting will start as an open meeting including the PI and any co-investigators and study personnel, where the PI will present the safety and efficacy materials for review to the DSMB and be available to answer any and all questions from the Board. The meeting will then be conducted as a closed meeting of the DSMB members for any further discussion and voting as to whether the study protocol should continue or be placed on hold with 2/3 votes being required for a recommendation. In addition to study safety and efficacy data, the DSMB will consider participant recruitment, accrual and retention, participant risk versus benefit, and any scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study. The research coordinator will keep minutes on the open meeting, and the Board chair will keep minutes on the closed meeting; the research coordinator will compile both sets of minutes and the recommendation into a report for approval by the DSMB prior to submission to the Principal Investigator and the IRB.

8 Study Administration, Data Handling and Record Keeping

8.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study;
- Who will have access to that information and why;
- Who will use or disclose that information; and
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the PI, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

8.2 Data Collection and Management

The University of Pennsylvania will be the only site where the research will be performed. Screening visits will involve the documentation of medical history, vital signs, and objective physical examination findings, and collection of blood and urine specimens as occurs routinely during standard care for islet transplant recipients. Medical history, laboratory, and other reports will be reviewed in the electronic medical record (EPIC®) or upon written consent of the potential study participant to obtain outside medical records. Specimens will consist of blood and urine samples, to be assayed for metabolic and hematological parameters, and to exclude pregnancy. Clinical laboratory test results may be made available to each subject's personal physicians upon their consent and written release of information. Additional data collected will include glucometer downloads, hypoglycemia questionnaires, adverse event logs, and symptom questionnaires and metabolic response data derived from the hyperinsulinemic euglycemic-hypoglycemic clamp experiments. Blood drawn including standard of care testing will not exceed 450 ml in any six-week period. Data culled from source documents will be transferred directly to a password protected database containing only the subject identification number. Subject ID numbers are assigned sequentially to each participant in each group and these numbers rather than names are used

to collect, store, and report participant information. None of the 18 biometric identifiers are entered into the database, however, date of birth, and dates of transplants, visits, and adverse events are maintained under lock and key in the source documents for monitoring and analysis purposes. Specimens that are archived or that may be sent out for future analyses are coded with subject ID numbers and do not contain any of the 18 biometric identifiers.

8.3 Records Retention

The PI will retain all essential study documents for at least 2 years after acceptance of the final manuscript for publication related to this protocol.

9 Study Monitoring, Auditing, and Inspecting

9.1 Study Monitoring Plan

The PI will review all clinical evaluations performed on subjects in order to promptly follow-up or intervene as appropriate. Each subject's data will be reviewed for the presence of adverse events at the completion of each study visit by the research nurse practitioner, and weekly during the PI's lab team meeting. The PI or the research nurse practitioner will provide direct oversight during all hyperinsulinemic euglycemic-hypoglycemic clamp procedures. Information relevant to adverse events will be collected, graded, recorded, and reported in compliance with *International Conference on Harmonization (ICH) Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting* and *ICH E6: Guidelines for Good Clinical Practice (GCP)* and follow the *Terminology Criteria for Adverse Events (TCAE)*. Adverse and serious adverse events' reporting includes reporting to the Penn IRB and DSMB. Serious adverse events will be reported to the Penn IRB and medical monitor (DSMB chair) in the appropriate expedited fashions; all other adverse events will be reported annually to the Penn IRB and DSMB.

A study monitor independent from the study team will be assigned this protocol and will be responsible to complete the monitoring process. The monitor will be selected from the staff of Clinical Research Coordinators working for other investigators in Penn's Institute for Diabetes, Obesity & Metabolism. A CV for the monitor will be obtained and updated annually. The CV will be kept on file in the Regulatory Binder for this protocol to document the qualifications of the monitor. The monitor will receive a monitoring manual including each current protocol, most recent approved consent forms, CRFs, and the DSMP (please see **Attachments**). These materials will be reviewed during a monitor training session with the PI and research coordinators scheduled after IRB approval but before subject enrollment, and any updates will be reviewed as necessary at the time of monitoring visits (please see below). A check list containing the elements of study initiation as described in 21 CFR Part 50 and ICH GCPs will be completed by the study staff prior to the first monitoring visit and placed in the Regulatory Binder.

Enrollment will be complete when 12 subjects are anticipated to complete Group 1 study visits, 12 subjects are anticipated to complete the Group 2 study visit, and 12 subjects are anticipated to complete the Group 3 study visit. Monitoring visits will occur when approximately 25%, 50%, and 75% of the Group 1 subjects have been enrolled. A final monitoring visit will be conducted after 100% of the Group 1 subjects have been enrolled and completed study, and will also serve as the close-out monitoring visit under GCP. The CRFs for the first two subjects and for one in every cohort of 5 subjects thereafter will be 100% source data verified. If a greater than 10% error rate is noted during the data review, the monitor will source data verify 100% of the data on a larger sample at the following two monitoring visits. Also, all subjects who discontinue due to adverse events, lost to follow-up, or other reasons will be reviewed for key safety and efficacy data and 100% source data verified. All subjects will be 100% source data verified for informed consent, inclusion/exclusion criteria, and serious and non-serious adverse events. The monitor will review the Regulatory Binder at the time of each monitoring visit for completeness and will assure that the CRFs are being completed in a timely manner.

CRFs will be completed at each study visit jointly by the research coordinator and nurse practitioner or PI. Data collected at each visit will be signed off by either the research nurse practitioner or the PI. Completed CRFs will be maintained in individual subject binders and stored under double lock inside the

office of the research coordinator. Subject demographic, clinical, and research data will be entered by the research coordinator, research nurse practitioner, or research technician; the PI will be responsible for all data queries and database corrections.

9.2 Auditing and Inspecting

The PI will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. Investigation Drug Service pharmacy, Center for Human Phenomic Science, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

10 Ethical Considerations

This study is to be conducted in accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the PI before commencement of this study.

10.1 Risks

Risks of phentolamine and propranolol infusions
Group 1 subjects will undergo testing on up to three occasions under randomized double-blind conditions of phentolamine, propranolol, or placebo infusion. Administration of phentolamine may be associated with an increase in heart rate, a reduction in blood pressure, or both, or may predispose to orthostatic hypotension. Rarely, myocardial infarction and cerebrovascular accidents have been reported to occur in association with marked hypotensive episodes. Administration of propranolol may be associated with a decrease in heart rate, a reduction in blood pressure, or both, or may predispose to orthostatic hypotension. All experiments will be conducted with continuous heart rate monitoring using a clinical telemetry unit, and heart rate and automated blood pressure recordings will be made at least every 15 minutes. To minimize the risk for a change in heart rate or blood pressure from either the phentolamine or propranolol infusion, the blinded infusion will be reduced by 50% and then turned off and then resumed at 50% based on changes in heart rate > 20 bpm and for absolute rates < 50 bpm or > 100 bpm, and for changes in BP of > 20 mmHg or for absolute measures <90/60 mmHg or > 160/100 mmHg. Patients with a history of coronary artery, cerebrovascular or peripheral vascular disease will not receive the phentolamine condition and in addition, a screening electrocardiogram will be performed to exclude any cardiac defects such as conduction delays. Propranolol use has also been associated with bronchospasm, and is contraindicated in patients with a history of bronchial asthma. Subjects experiencing upper respiratory tract infections will be re-scheduled, and those with a history of bronchial asthma will not receive the propranolol condition.

Risks of study procedures
All three groups will undergo hyperinsulinemic (1 mU/kg·min for 3 hours) euglycemic (~ 90 mg/dl) followed by hypoglycemic (~ 50 mg/dl) clamp testing. These procedures include risks pertaining to blood draws, maintenance of intravenous catheters, and the delivery of test substances (insulin and glucose) during the metabolic clamp procedures. Peripheral blood draws performed during these research studies will not exceed 450 mL per six-week period; subjects with anemia will be excluded from participation. Blood draws and intravenous line placement for metabolic testing may cause the subject to experience some discomfort or bruising at the site of the needle/catheter entry. The risks of intravenous line placement for metabolic testing include bleeding, displacement, and interstitial infusion of fluids; rarely local vein thrombosis, infection or thrombophlebitis may develop. The administration of insulin intravenously during the clamp procedures may lead to a greater degree of hypoglycemia than expected,

but would be rapidly corrected with intravenous glucose. The stable isotope of glucose infused during the glycemic clamps is tested for identity, sterility, and absence of pyrogens by the Penn Investigational Drug Service, and so carries no additional risks. Insertion of the catheter for continuous glucose monitoring could cause bruising, bleeding, or rarely, infection. The conduct of these procedures in the Center for Human Phenomic Science by experienced research nurses and nurse practitioners greatly minimizes the risks of needle/catheter placement and administration of test substances.

10.2 Benefits

Islet transplant recipients may benefit from the medical assessments and close follow-up that participation requires. Society will benefit from an improved understanding of the mechanisms by which intrahepatic islet transplantation restores glucose counterregulation in recipients with a history of long standing type 1 diabetes and hypoglycemia unawareness that should inform the necessity of islet innervation and appropriate site for transplantation of novel cell therapy products currently in development for the treatment of diabetes.

10.3 Risk Benefit Assessment

To the extent that the risks of participation have been minimized by the careful selection of exclusion criteria and implementation of procedural and protocol monitoring plans, the risks of participating in the study are outweighed by the potential benefits of participating in the study that is designed to elucidate the mechanisms by which islet transplantation may protect patients from the development of hypoglycemia. The alternative to participation is not to participate.

10.4 Informed Consent Process / HIPAA Authorization

Subjects recruited or referred for participation will be subjected to a structured telephone interview conducted by one of the research coordinators to determine potential eligibility; in some cases candidates' medical records may be reviewed by the PI after a signed release of medical records is received. If review of the phone interview and any medical records indicates that the potential participant is not eligible, s/he will be notified by the research coordinator and explained why this is so; if review indicates that the potential participant may be eligible, s/he will be contacted by the research coordinator to schedule the screening visit and the informed consent form will be mailed to subject for review.

At the screening visit, the study details and procedures will be discussed with a research coordinator and at least one of the PI or research nurse practitioner. The potential participant is given adequate time to ask questions and review the informed consent document. Once satisfied that all questions have been answered by the PI or research nurse practitioner, the potential participant will either decline to participate or sign the informed consent document. This may occur at a subsequent visit if the potential participant desires, in order to think further about what participation means and/or to consult with family, friends and/or a personal physician. The consent form is signed in the presence of a witness (research coordinator +/- family member). All participants must read, sign, and date a consent form before entering the study, undergoing physical examination or undergoing any testing.

The informed consent form will be revised whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect participation in the study. All investigators and research coordinators involved in this study have current patient-oriented research certification. The PI has completed a Master's program in Translation Research that included formal coursework in Research Ethics at the University of Pennsylvania.

11 Study Finances

11.1 Funding Source

This study is financed through Public Health Services research grant 2R01-DK-091331 to the PI from the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health.

11.2 Conflict of Interest

No participating investigator has a conflict of interest with this study. All investigators will follow the University of Pennsylvania Policy on Conflicts of Interest Related to Research.

11.3 Subject Stipends or Payments

Subjects will receive $50 compensation for the screening visit, and $150 compensation for each CHPS study visit for hyperinsulinemic euglycemic-hypoglycemia clamp testing, as well as reimbursement for parking and travel related expenses in order to encourage study completion. No compensation will be provided for injury that may be incurred as a result study participation.

12 Publication Plan

Publication and presentation of the data derived from this protocol will be the responsibility of the PI. Neither the complete nor any part of the results of the study carried out under this protocol will be published or passed on to any third party without the consent of the PI. All referring physicians from collaborating institutions will be invited to participate as co-authors for presentations and publications that involve their patients. In the case that participants for either Group 2 or Group 3 are derived mostly from a collaborating institution, the referring physician will be invited to lead authorship of presentations and publications comparing that Group to the Group 1 placebo condition with the PI serving as senior author.

13 References

(2015). Glycemic Targets. Diabetes Care *38*, S33-S40.

Abrahamsson,N., Borjesson,J.L., Sundbom,M., Wiklund,U., Karlsson,F.A., and Eriksson,J.W. (2016). Gastric Bypass Reduces Symptoms and Hormonal Responses in Hypoglycemia. Diabetes *65*, 2667-2675.

Auer,R.N. (2004). Hypoglycemic brain damage. Metabolic Brain Disease *19*, 169-175.

Bellin,M.D., Parazzoli,S., Oseid,E., Bogachus,L.D., Schuetz,C., Patti,M.E., Dunn,T., Pruett,T., Balamurugan,A.N., Hering,B., Beilman,G., Sutherland,D.E., and Robertson,R.P. (2014). Defective Glucagon Secretion During Hypoglycemia After Intrahepatic But Not Nonhepatic Islet Autotransplantation. Am. J. Transplant *14*, 1880-1886.

Bernroider,E., Brehm,A., Krssak,M., Anderwald,C., Trajanoski,Z., Cline,G., Shulman,G.I., and Roden,M. (2005). The role of intramyocellular lipids during hypoglycemia in patients with intensively treated type 1 diabetes. Journal of Clinical Endocrinology and Metabolism *90*, 5559-5565.

Boden,G., Chen,X.H., Desantis,R., Kolaczynski,J., and Morris,M. (1993). Evidence That Suppression of Insulin-Secretion by Insulin Itself Is Neurally-Mediated. Metabolism-Clinical and Experimental *42*, 786-789.

Bruin,J.E., Rezania,A., Xu,J., Narayan,K., Fox,J.K., O'Neil,J.J., and Kieffer,T.J. (2013). Maturation and function of human embryonic stem cell-derived pancreatic progenitors in macroencapsulation devices following transplant into mice. Diabetologia *56*, 1987-1998.

Choudhary,P., Rickels,M.R., Senior,P.A., Vantyghem,M.C., Maffi,P., Kay,T.W., Keymeulen,B., Inagaki,N., Saudek,F., Lehmann,R., and Hering,B.J. (2015). Evidence-Informed Clinical Practice Recommendations for Treatment of Type 1 Diabetes Complicated by Problematic Hypoglycemia. Diabetes Care *38*, 1016-1029.

Cooperberg,B.A. and Cryer,P.E. (2010). Insulin reciprocally regulates glucagon secretion in humans. Diabetes *59*, 2936-2940.

Cryer,P.E. (2008). The Barrier of Hypoglycemia in Diabetes. Diabetes *57*, 3169-3176.

Cryer,P.E. (2013). Mechanisms of Hypoglycemia-Associated Autonomic Failure in Diabetes. New England Journal of Medicine *369*, 362-372.

Cryer,P.E., Davis,S.N., and Shamoon,H. (2003). Hypoglycemia in diabetes. Diabetes Care *26*, 1902-1912.

Feltbower,R.G., Bodansky,H.J., Patterson,C.C., Parslow,R.C., Stephenson,C.R., Reynolds,C., and McKinney,P.A. (2008). Acute complications and drug misuse are important causes of death for children and young adults with type 1 diabetes - Results from the Yorkshire Register of Diabetes in Children and Young Adults. Diabetes Care *31*, 922-926.

Gardemann,A., Jungermann,K., Grosse,V., Cossel,L., Wohlrab,F., Hahn,H.J., Blech,W., and Hildebrandt,W. (1994). Intraportal Transplantation of Pancreatic-Islets Into Livers of Diabetic Rats - Reinnervation of Islets and Regulation of Insulin-Secretion by the Hepatic Sympathetic-Nerves. Diabetes *43*, 1345-1352.

Gerich,J.E., Lorenzi,M., Tsalikian,E., and Karam,J.H. (1976). Studies on Mechanism of Epinephrine-Induced Hyperglycemia in Man - Evidence for Participation of Pancreatic Glucagon-Secretion. Diabetes *25*, 65-71.

Girdler,S.S., Hinderliter,A.L., and Light,K.C. (1993). Peripheral Adrenergic-Receptor Contributions to Cardiovascular Reactivity - Influence of Race and Gender. Journal of Psychosomatic Research *37*, 177-193.

Lin,Y.K., Faiman,C., Walsh,R.M., Stevens,T., Bottino,R., and Hatipoglu,B.A. (2016). Spontaneous Hypoglycemia after Islet Autotransplantation for Chronic Pancreatitis. J. Clin. Endocrinol. Metab jc20162111.

Lind,M., Svensson,A.M., Kosiborod,M., Gudbjornsdottir,S., Pivodic,A., Wedel,H., Dahlqvist,S., Clements,M., and Rosengren,A. (2014). Glycemic Control and Excess Mortality in Type 1 Diabetes. New England Journal of Medicine *371*, 1972-1982.

Luzi,L., Battezzati,A., Perseghin,G., Bianchi,E., Vergani,S., Secchi,A., Larocca,E., Staudacher,C., Spotti,D., Ferrari,G., DiCarlo,V., and Pozza,G. (1992). Lack of Feedback Inhibition of Insulin-Secretion in Denervated Human Pancreas. Diabetes *41*, 1632-1639.

Mccoy,R.G., Van Houten,H.K., Ziegenfuss,J.Y., Shah,N.D., Wermers,R.A., and Smith,S.A. (2012). Increased Mortality of Patients With Diabetes Reporting Severe Hypoglycemia. Diabetes Care *35*, 1897-1901.

Miller,K.M., Foster,N.C., Beck,R.W., Bergenstal,R.M., Dubose,S.N., DiMeglio,L.A., Maahs,D.M., and Tamborlane,W.V. (2015). Current State of Type 1 Diabetes Treatment in the U.S.: Updated Data From the T1D Exchange Clinic Registry. Diabetes Care *38*, 971-978.

Pagliuca,F.W., Millman,J.R., Gurtler,M., Segel,M., Van Dervort,A., Ryu,J.H., Peterson,Q.P., Greiner,D., and Melton,D.A. (2014). Generation of Functional Human Pancreatic beta Cells In Vitro. Cell *159*, 428-439.

Pedersen-Bjergaard,U., Pramming,S., Heller,S.R., Wallace,T.M., Rasmussen,A.K., Jorgensen,H.V., Matthews,D.R., Hougaard,P., and Thorsteinsson,B. (2004). Severe hypoglycaemia in 1076 adult patients with type 1 diabetes: influence of risk markers and selection. Diabetes Metab Res. Rev. *20*, 479-486.

Rezania,A., Bruin,J.E., Arora,P., Rubin,A., Batushansky,I., Asadi,A., O'Dwyer,S., Quiskamp,N., Mojibian,M., Albrecht,T., Yang,Y.H.C., Johnson,J.D., and Kieffer,T.J. (2014). Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. Nature Biotechnology *32*, 1121-1133.

Rickels,M.R., Fuller,C., Dalton-Bakes,C., Markmann,E., Palanjian,M., Cullison,K., Tiao,J., Kapoor,S., Liu,C.Y., Naji,A., and Teff,K.L. (2015). Restoration of Glucose Counterregulation by Islet Transplantation in Long-standing Type 1 Diabetes. Diabetes *64*, 1713-1718.

Rickels,M.R., Kong,S.M., Fuller,C., Dalton-Bakes,C., Ferguson,J.F., Reilly,M.P., Teff,K.L., and Naji,A. (2014). Insulin sensitivity index in type 1 diabetes and following human islet transplantation: comparison of the minimal model to euglycemic clamp measures. American Journal of Physiology-Endocrinology and Metabolism *306*, E1217-E1224.

Rickels,M.R., Liu,C., Shlansky-Goldberg,R.D., Soleimanpour,S.A., Vivek,K., Kamoun,M., Min,Z., Markmann,E., Palangian,M., Dalton-Bakes,C., Fuller,C., Chiou,A.J., Barker,C.F., Luning Prak,E.T., and Naji,A. (2013). Improvement in beta-cell secretory capacity after human islet transplantation according to the CIT07 protocol. Diabetes *62*, 2890-2897.

Rickels,M.R., Pelekis,A.J., Markmann,E., Dalton-Bakes,C., Kong,S.M., Teff,K.L., and Naji,A. (2016). Long-term improvement in glucose control and counterregulation by islet transplantation for type 1 diabetes. J. Clin. Endocrinol. Metab jc20161649.

Robertson,R.P. (2015). Puzzling About Partial Glucagon Responses to Hypoglycemia in Intrahepatic Islet Recipients: Missing Pieces. Diabetes *64*, 1511-1512.

Skrivarhaug,T., Bangstad,H.J., Stene,L.C., Sandvik,L., Hanssen,K.F., and Joner,G. (2006). Long-term mortality in a nationwide cohort of childhood-onset type 1 diabetic patients in Norway. Diabetologia *49*, 298-305.

Stadler,M., Peric,S., Strohner-Kaestenbauer,H., Kramar,R., Kaestenbauer,T., Reitner,A., Auinger,M., Kronenberg,F., Irsigler,K., Amiel,S.A., and Prager,R. (2014). Mortality and Incidence of Renal Replacement Therapy in People With Type 1 Diabetes Mellitus-A Three Decade Long Prospective Observational Study in the Lainz T1DM Cohort. Journal of Clinical Endocrinology & Metabolism *99*, 4523-4530.

Tanenberg,R.J., Newton,C.A., and Drake,A.J. (2010). Confirmation of hypoglycemia in the "dead-in-bed" syndrome, as captured by a retrospective continuous glucose monitoring system. Endocr. Pract. *16*, 244-248.

Tattersall,R.B. and Gill,G.V. (1991). Unexplained Deaths of Type-1 Diabetic-Patients. Diabetic Medicine *8*, 49-58.

Towler,D.A., Havlin,C.E., Craft,S., and Cryer,P. (1993). Mechanism of Awareness of Hypoglycemia - Perception of Neurogenic (Predominantly Cholinergic) Rather Than Neuroglycopenic Symptoms. Diabetes *42*, 1791-1798.

Tsai,E.B., Sherry,N.A., Palmer,J.P., and Herold,K.C. (2006). The rise and fall of insulin secretion in type 1 diabetes mellitus. Diabetologia *49*, 261-270.

Tu,E., Twigg,S.M., and Semsarian,C. (2010). Sudden death in type 1 diabetes: the mystery of the 'dead in bed' syndrome. Int. J Cardiol. *138*, 91-93.

White,N.H., Gingerich,R.L., Levandoski,L.A., Cryer,P.E., and Santiago,J.V. (1985). Plasma Pancreatic-Polypeptide Response to Insulin-Induced Hypoglycemia As A Marker for Defective Glucose Counterregulation in Insulin-Dependent Diabetes-Mellitus. Diabetes *34*, 870-875.

Wolfe,R.R. and Chinkes,D.L. (2005). Isotope Tracers in Metabolic Research: Principles and Practice of Kinetic Analysis. (Hoboken, NJ: John Wiley & Sons, Inc.).

Zhou,H., Zhang,T., Bogdani,M., Oseid,E., Parazzoli,S., Vantyghem,M.C., Harmon,J., Slucca,M., and Robertson,R.P. (2008). Intrahepatic glucose flux as a mechanism for defective intrahepatic islet alpha-cell response to hypoglycemia. Diabetes *57*, 1567-1574.

14 Attachments

- Data and Safety Monitoring Plan
- Informed Consent Form – Group 1
- Informed Consent Form – Group 2
- Informed Consent Form – Group 3
- Phentolamine (Regitine®) Package Insert
- Propranolol Package Insert