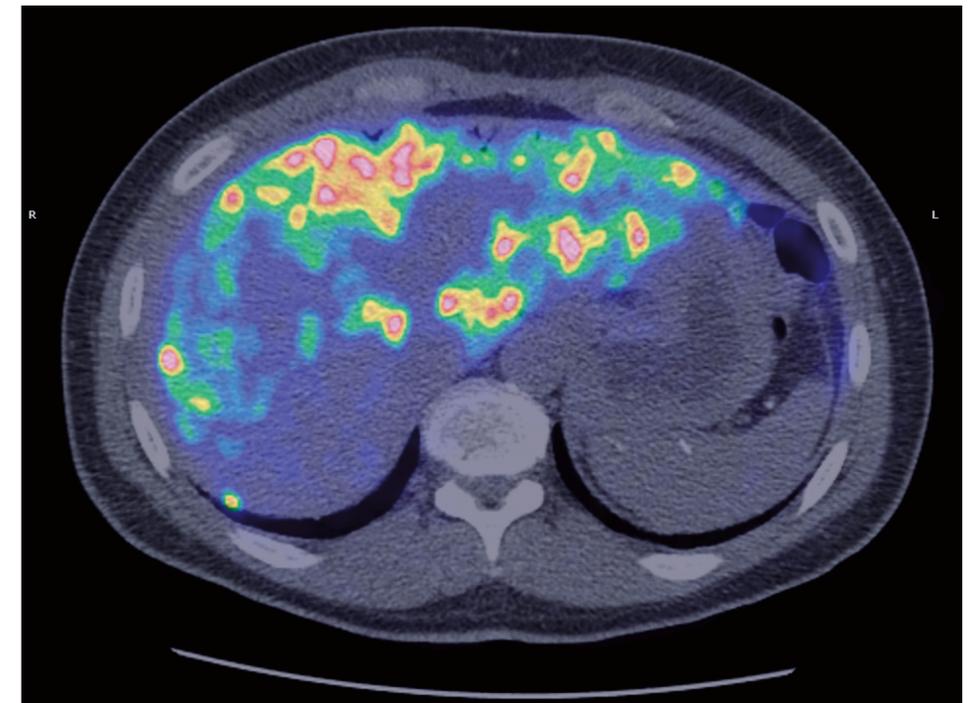


Thesis for doctoral degree (Ph.D.)  
2010

# EVALUATION OF STRATEGIES TO PREDICT AND IMPROVE EARLY GRAFT SURVIVAL IN CLINICAL ISLET TRANSPLANTATION



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CLINICAL ISLET TRANSPLANTATION

Torbjörn Lundgren

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From  
THE DEPARTMENT OF CLINICAL SCIENCE,  
INTERVENTION AND TECHNOLOGY (CLINTÉC)  
DIVISION OF TRANSPLANTATION SURGERY  
Karolinska Institutet, Stockholm, Sweden

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TRANSPLANTATION**

Torbjörn Lundgren



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**To my family,  
Annika, Samuel, Joel and Vilgot**

## ABSTRACT

Transplantation of the islets of Langerhans as a treatment for type 1 diabetes (T1DM) is a procedure still under development. For the last decade it has been possible to obtain insulin independence by repeated infusions of islets. However, most patients must resume insulin therapy in 2-3 years. The side effects associated with today's immunosuppressive medication limit the procedure to patients with severe and frequent episodes of hypoglycemia or to T1DM patients who need immunosuppressive medication for other reasons, e.g. due to previous kidney transplantation. The efficacy and long term results of islet transplantation need to be improved for the treatment to be considered for also other categories of patients.

In this thesis a new technique, the two-layer method (TLM), to avoid hypoxic damage to the pancreas during the period of cold storage between donor operation and islet isolation was evaluated. PET/CT was introduced in a preclinical large animal model of islet transplantation and thereafter used to study the early post-transplantation phase of clinical islet transplantation. Finally, a new ratio,  $\Delta CP/GCr$ , was used to evaluate short term outcomes of repeated clinical islet transplantations.

No significant differences could be seen in islet isolation yield or islet transplantation outcomes using TLM compared to conventional cold storage. When utilizing PET/CT to monitor the transplantation of [ $^{18}F$ ]-FDG labeled islets, these were heterogeneously spread in the liver, with fractions of the graft found in "hot spots". At least 25 % of the radioactivity contained within the islets was lost during the transplantation indicating significant early damage to the graft, possibly due to the instant blood mediated inflammatory reaction (IBMIR). Evaluating the clinical outcome of 110 transplantations, we showed that a large islet graft, a high stimulation index and short cold ischemia time (CIT) were factors that significantly improved clinical outcome. No correlation was found between initial renal graft function and the outcome of islet transplantation from the same donor.

This thesis has investigated areas where improvements must be made for islet transplantation to reach higher efficacy and become a treatment modality for more patients. The need for repeated islet infusions has made it difficult to study the effect of each islet preparation. Tools have here been introduced to facilitate this individual evaluation. Factors that predict the clinical outcome have been identified. Findings include the importance of CIT. New methods to preserve the pancreas before isolation are needed to improve outcome of both isolation and transplantation. A large fraction of islets implanted is lost during transplantation. The presented PET/CT technique may be used to further characterize early post-transplantation events and can become an important instrument in evaluation of new methods to enhance initial islet survival.

Keywords: Islets of Langerhans, clinical islet transplantation, cold storage, outcomes, PET/CT, imaging, IBMIR.

## LIST OF PUBLICATIONS

- I. **No Beneficial Effect of Two-Layer Storage Compared With UW-Storage on Human Islet Isolation and Transplantation**  
José Caballero-Corbalán, Torsten Eich, [Torbjörn Lundgren](#), Aksel Foss, Marie Felldin, Ragnar Källén, Kaija Salmela, Annika Tibell, Gunnar Tufveson, Olle Korsgren, Daniel Brandhorst (Transplantation 2007;84:864-869)
- II. **Positron Emission Tomography: A Real-Time Tool to Quantify Early Islet Engraftment in a Preclinical Large Animal Model**  
Torsten Eich, Olof Eriksson, Anders Sundin, Sergio Estrada, Daniel Brandhorst, Heide Brandhorst, Bengt Långström, Bo Nilsson, Olle Korsgren, [Torbjörn Lundgren](#) (Transplantation 2007;84: 893–898)
- III. **Visualization of Early Engraftment in Clinical Islet Transplantation by Positron-Emission Tomography**  
Torsten Eich, Olof Eriksson, [Torbjörn Lundgren](#)  
(New England Journal of Medicine 2007 Jun 28;356(26):2754-5)
- IV. **Positron Emission Tomography in Clinical Islet Transplantation**  
Olof Eriksson, Torsten Eich, Anders Sundin, Annika Tibell, Gunnar Tufveson, Helene Andersson, Marie Felldin, Aksel Foss, Lauri Kyllönen, Bengt Långström, Bo Nilsson, Olle Korsgren, [Torbjörn Lundgren](#)  
(American Journal of Transplantation 2009; 9: 2816–2824)
- V. **Can the Outcome of Clinical Islet Transplantations be Predicted Pretransplant?**  
[Torbjörn Lundgren](#), Andrew Friberg, Helene Andersson, Marie Felldin, Trond Jenssen, Lauri Kyllönen, Gunnar Tufveson, Bo Nilsson, Olle Korsgren, Annika Tibell. (Manuscript)

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## LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BW	Body weight
CIT	Cold ischemia time
CP/G	C-peptide to glucose ratio
CP/GCr	C-peptide to the product of glucose and creatinine ratio
CT	Computed Tomography
FDG	Fluoro-Deoxy-Glucose
GFR	Glomerular Filtration Rate
GSIS	Glucose-Stimulated Insulin Secretion
HTK	Histidine–Tryptophan–Ketoglutarate
IA	Islet Alone
IAK	Islet After kidney
IBMIR	Instant Blood Mediated Inflammatory Reaction
IEQ	Islet Equivalents
i.v.	intra-venous
MRI	Magnetic Resonance Imaging
PET	Positron Emission Tomography
PFD	Perfluorodecalin
RAS	Ringer’s Acetate Solution
s.c.	sub-cutaneous
SEM	Standard Error of the Mean
SI	Stimulation Index
SIK	Simultaneous Islet and Kidney Transplantation
T1DM	Type 1 Diabetes Mellitus
TLM	Two-Layer Method
UWS	University of Wisconsin Solution



# **1 BACKGROUND**

## **1.1 DIABETES**

### **1.1.1 History**

Diabetes, defined as thirst and large volumes of sweet smelling urine, has been a known entity for centuries. It was, however, not until the late 1880s that von Mering and Minkowski showed that diabetes could be induced in dogs by removing the pancreas (1). The cause of this fatal illness was thereafter sought for in this gland. Banting and Best isolated insulin in 1921 and proved that the lack of this enzyme, produced in the islets of Langerhans, was the cause of diabetes (2). Treatment results improved dramatically using insulin. In 1960, Yalow and Berson introduced a radio-immunological method for measuring insulin and physicians could then thereby separate juvenile diabetes (Type 1) with low or no insulin levels from adult diabetes (Type 2) with normal or high insulin levels (3). This thesis is focused on Type 1 Diabetes Mellitus (T1DM) where the insulin production capacity has been lost and could be replaced by transplantation.

### **1.1.2 Type 1 Diabetes Mellitus**

The underlying cause and trigger of T1DM is still unknown. However, an auto-immune destruction of the islets is involved in the process and auto-antibodies can be found in about 80% of newly diagnosed T1DM patients. In homozygotic twins, where one of them has T1DM, it is a 30-50% risk that the other twin will develop the disease. This shows that not only genetic, but also environmental factors are involved. As a consequence of insulin deficiency the carbohydrate metabolism becomes severely affected. This is shown by hyperglycemia and high concentrations of glycerol, fatty acids and ketone bodies. Distribution of T1DM is uneven throughout the world, with

the highest incidence in developed countries far from the equator like Sweden and Finland (Figure 1) (4). In Sweden it is estimated that ~50 000 (one in every 180) persons have T1DM today.

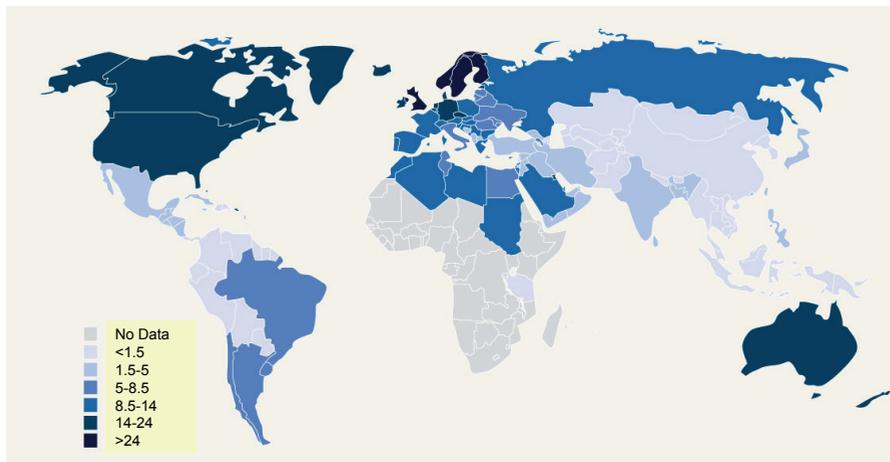


Figure 1. New cases of type 1 diabetes mellitus in children, 0-14 years, predicted 2010 (per 100 000 children 0-14 years). Source; IDF Diabetes Atlas 4<sup>th</sup> Edition, 2009.

#### 1.1.2.1 Treatment options for T1DM today

The introduction of insulin revolutionized the treatment of diabetes from the 1920's and onward. Various insulins with different distribution methods is still the only treatment option available for most T1DM patients. The medication has been individualized and fine tuned over decades. Self measuring of blood glucose and a combination of long and short acting subcutaneously (s.c.) administered insulin is now the standard treatment. All insulins available on the Swedish market are made from recombinant human insulin with different carriers allowing quick or delayed release to the bloodstream. Some patients prefer administration of insulin through a pump s.c. instead of separate injections. Beta cell replacement therapy can be indicated for a selected group of patients, see 1.2 and onwards.

### *1.1.2.2 Complications in T1DM*

Insulin treatment has made it possible to survive with T1DM. However, long term complications of the disease still constitute a major problem. Hyperglycemia, in the long run, leads to microangiopathy developing in the eyes (retinopathy), renal glomeruli (nephropathy) and peripheral nerves (neuropathy). These conditions may lead to blindness, uremia and amputations (due to poor wound healing). Clinical studies have shown the close relationship between the level of metabolic control and the development of these complications (5). A well controlled HbA1c resulted in less long term complications, but increased the risk for hypoglycemia. This is one of the main problems in treatment of T1DM patients today, the increasing frequency of hypoglycemia when trying to help the patient to a better long term prognosis (6).

## **1.2 BETA CELL REPLACEMENT THERAPY IN TYPE 1 DIABETES MELLITUS**

Over the last 50 years, transplantation has emerged as the treatment of choice for a wide range of diseases. Today thousands of kidneys, livers, hearts, lungs and pancreases are transplanted each year and the number of transplant centers is increasing worldwide. Often the transplantations serve to replace an organ where many of the functions and morphological integrity have been lost (i.e. liver cirrhosis or polycystic kidney disease). However, there are several examples where only a part of an organ's repertoire of functions is distorted. Such indications include liver transplantation with the sole purpose to enable the patient to produce a hormone or enzyme (7, 8) or when the whole bone marrow is replaced because of one malignant cell line, failure to synthesize hemoglobin or for various immunodeficiencies (9).

In T1DM, the failure of one type of cell causes a systemic disease that, without replacing the hormone, i.e. insulin, leads to death. Even when treated with insulin the

patients suffer because of the lack of the minute to minute feedback system that normally is provided by the  $\beta$  cells, T1DM in the long run may lead to blindness, amputations, renal failure and premature death. It is today widely accepted that the angiopathy leading to these complications is a result of the inferior metabolic control of today's insulin administration regimes in comparison with that in a non-diabetic person. Taking this into account,  $\beta$  cell replacement therapy through transplantation could play an important role. The provision of a sufficient  $\beta$  cell mass can fine tune blood glucose through the production and release of hormones in a physiological manner, restoring normoglycemia, and avoiding long term complications.

The main hurdle in the advancement of transplantation in general has been how to evade the allogenic barrier of the immune system. In any transplantation between two individuals immunosuppressant drugs are needed to avoid rejection of the transplant (see 1.2.2). Another hurdle to take into account, when replacing the destroyed cells in the treatment of T1DM, is the autoimmune process involved in the etiology of the disease.

The  $\beta$  cells of a human are spread within the Islets of Langerhans, which themselves are spread diffusely within the pancreas. The total volume of the islets is about 1-2 mL and represents only 1-2% of the pancreas tissue. When considering  $\beta$  cell replacement therapy there are today two options; either to replace the whole organ, pancreas transplantation, or to prior to transplantation separate the islets of Langerhans from the exocrine tissue, islet transplantation.

Islet transplantation has several theoretical advantages compared to whole organ transplantation. It's a minimally invasive treatment, islets can be pretreated to avoid

rejection or to document and enhance performance. In the future islets could potentially be derived from stem cells or animals (xenotransplantation) securing  $\beta$  cell availability (10).

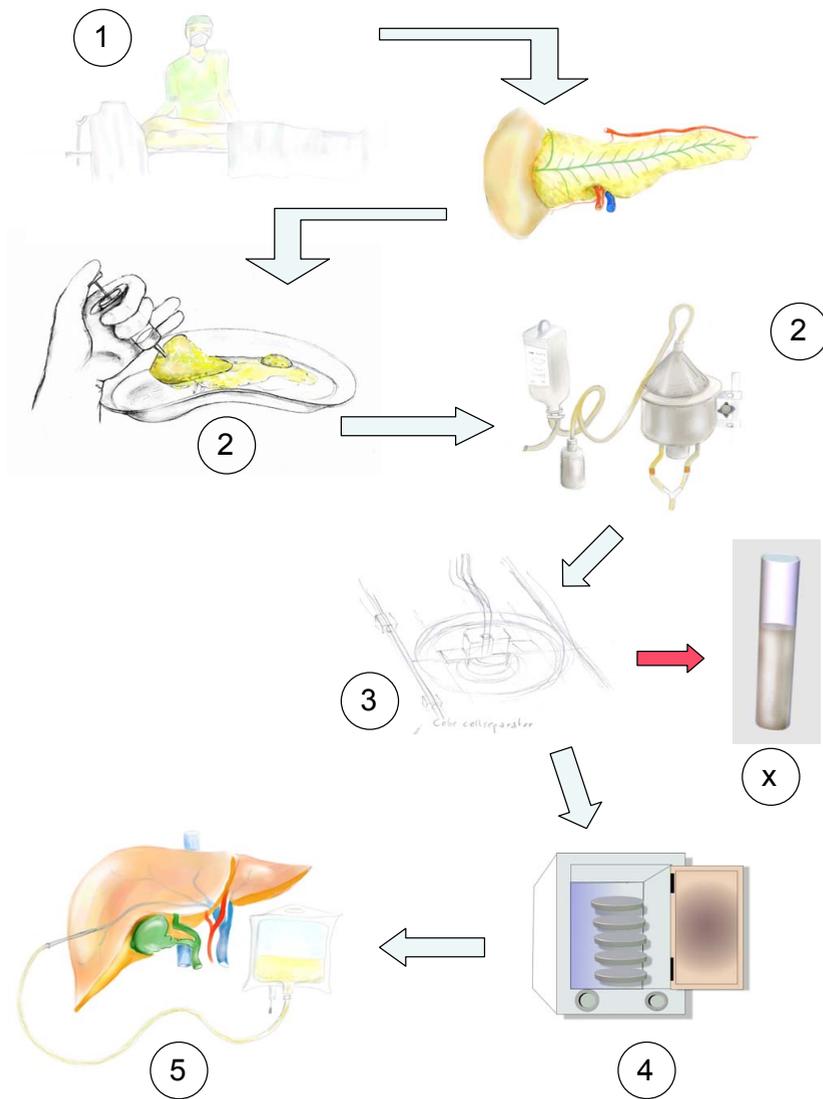
### **1.2.1 History**

The first series of pancreas transplantations was performed by Lillihei, Kelly and co-workers at the University of Minnesota in the 1960's (11). Today more than 23 000 pancreas transplantations have been reported to the International Pancreas Transplant Registry (12). Advancements in organ procurement, surgical technique and immunosuppressive medication have improved results over time. The most common technique today places the new pancreas in the abdomen with arterial vascular supply coming from the recipient's iliac artery. Venous drainage of the graft can be led to either the portal vein or systemic system. Commonly a piece of the donor's duodenum is transplanted with the graft and is anastomosed to the recipient's jejunum to drain the pancreatic exocrine secretions. The recipient's own pancreas is left in place.

Combined pancreas/kidney transplantation is a widely accepted therapy for T1DM patients with end stage renal disease. At three years after transplantation 84.7 % of the patients are insulin independent with functioning kidney grafts (13). Corresponding results with pancreas transplantation alone or transplantation performed after kidney transplantation with grafts from different donors are 74.9 and 78.0% respectively.

Pancreas transplantation is considered to be a major surgical procedure and short term complications include cardiac morbidity, pancreatitis, intra abdominal infections and graft loss due to thrombosis. Ironically, the complications of pancreas transplantation emanates from the exocrine portion that only serves as a carrier for the endocrine tissue.

Minimally invasive methods to transplant isolated islets have been developed in parallel with the clinical advancements of pancreas transplantation. Already in 1972 Lacy and co-workers (14) could cure chemically induced diabetes in rats through intraabdominal or intraportal transplantation of isolated islets. To isolate large numbers of islets from human pancreases proved to be more difficult. The inability to transplant enough viable islets hampered the possibilities to perform clinical trials. In 1988 Ricordi introduced an islet isolation technique that improved outcomes of human islet isolation (15) and in 1990, the St Louis group reported the first insulin independent type 1 diabetic recipient transplanted with islets from two deceased donors (16). Exogenous insulin however had to be reinstated on day 25 after transplantation, probably due to rejection (Figure 2). Warnock and colleagues 1992 published the first case with a patient remaining insulin independent more than a year after transplantation (17). The patient received islets from a total of 5 donors equaling about 10 000 islet equivalents (IEQ) per kilogram bodyweight. Results slowly improved but were clearly inferior to those of pancreas transplantation. Still at the end of the last millennium insulin independence rates after islet transplantation were, at one year, only about 10-15% (ITR Giessen [www.med.uni-giessen.de/itr](http://www.med.uni-giessen.de/itr)).



John Sandberg

Figure 2. Clinical islet transplantation. The pancreas is procured from a deceased donor (1) and is transported to the islet isolation facility where it undergoes mechanical and enzymatical digestion (2). This is followed by a separation of endocrine and exocrine (x) tissue by centrifugation (3). Islets can thereafter be stored in culture (4) awaiting the transplantation (5). Today intraportal infusion via a transhepatic, percutaneously placed, catheter is the most common technique for islet transplantation.

### **1.2.2 Immunosuppressive medication**

In any transplantation between individuals (allotransplantation) it's necessary to give immunosuppressive medication to avoid rejection. The only exception to this rule is transplantation between identical twins. When transplanting islets to treat T1DM, the underlying autoimmunity of the disease must also be considered. If not, diabetes can reoccur in the transplanted tissue (18-20). The importance of autoantibody titres is controversial. There are reports indicating inferior results in pretransplant autoantibody positive patients, but others have seen no such correlation (21, 22). However, in most cases the same immunosuppression used to avoid alloimmunity seems to keep autoimmunity at bay.

The immunosuppressive drugs used can be divided into induction therapy that is given for a short period in conjunction to the transplant and maintenance therapy that the patient has to use during the lifespan of the graft.

For maintenance, combinations of drugs are given. This is both to limit the side effects of high doses of any specific drug and to benefit from the different mechanisms of action between the drugs. Introduction of the first calcineurin inhibitor, cyclosporine A, in the late 1970s helped to improve short term results in all types of organ transplantation. Triple treatment with cyclosporine A, azathioprine (a proliferation inhibitor) and steroids became the main elements of all transplantation protocols for many years. In the last ten years tacrolimus (a second generation calcineurin inhibitor) to a large extent has replaced cyclosporine A and mycophenolate acid has replaced azathioprine.

Sirolimus was introduced in 2000. This was the first drug in a new category (mTOR inhibitors) also targeting IL-2, but at a different level and considered less nephrotoxic

than both cyclosporine A and tacrolimus. It was thought that sirolimus would be able to replace the calcineurin inhibitors. This hope has, unfortunately, not been fulfilled. Sirolimus is today used in combination with other drugs. It is commonly used in islet transplantation protocols and facilitated to start to transplant without the use of glucosteroids. However, there are concerns about effects on  $\beta$ -cell adaptation to hyperglycemia (23) and in vivo proliferation of  $\beta$ -cells (24) using the drug.

The pancreas is considered more prone to rejection than many other organs. Induction therapy with lymphocyte depleting antibodies is common. In islet transplantation the trend is currently shifting from IL-2 receptor blockers (daclizumab and basiliximab) that were used in the original Edmonton protocol (25) to depleting antibodies (anti thymocyte globulin) ([www.citregistry.org](http://www.citregistry.org)).

Some specific side effects of immunosuppressive drugs are found in tables 1 and 2. It is certainly a problem when the main elements of treatment are calcineurin inhibitors (Cyclosporine A, Tacrolimus) that have specific side effects influencing the transplanted graft, nephrotoxicity (26) in renal transplantation and disturbed glucose metabolism in pancreas/islet transplantation (27).

The therapy as such carries important general side effects that are not specific for any drugs, but rather related to the level of total immunosuppression. This involves all types of infections (bacterial, viral and fungal). Infectious complications are most common early after transplantation when the levels of the drugs are high (28). Through the years clinicians have become more acquainted with the present therapies and generally the recommended trough levels of the calcineurin inhibitors have been lowered. Severe infections are less common today than 10 or 15 years ago.

<b>Generic name</b>	<b>Brand names</b>	<b>Introduced</b>	<b>Mechanism of action</b>	<b>Specific side effects</b>
Prednisolone	Prednisone®	1950	Anti-inflammatory	Osteoporosis, Insulin resistance
Azathioprine	Imuran® Imurel®	1960	Limits expansion of White blood cells	Neutropenia
Cyclosporine A	Sandimmune® Neoral®	1979	Inhibits T cell proliferation Calcineurin inhibitor	Nephrotoxicity, Hypertension
Tacrolimus	Prograf® Advagraf®	1995	Inhibits T cell proliferation Calcineurin inhibitor	Nephrotoxicity, Diabetes
Mycophenolate acid	CellCept® Myfortic®	1995 2004	Limits expansion of white blood cells (lymphocytes)	Upper GI symptoms, Neutropenia.
Sirolimus Everolimus	Rapamune® Certican®	2000 2003	Inhibits T cell proliferation by blocking intracellular signaling	Impairs wound healing, Mouth ulcers, Interstitial alveolitis, Hyperlipidemia

Table 1. Common drugs in maintenance therapy

<b>Generic name</b>	<b>Brand names</b>	<b>Introduced</b>	<b>Mechanism of action</b>	<b>Specific side effects</b>
Methyl prednisolone	Solu-Medrol®	1950	Anti Inflammatory	Osteoporosis, Insulin resistance
Daclizumab	Zenapax®	1999	IL-2 receptor blockers	
Basiliximab	Simulect®	1999		
Anti Thymocyte Globuline	ATG-Fresenius® Thymoglobuline®	1999 2002	Depletes T-lymphocytes	Allergic reactions, neutropenia

Table 2. Common drugs in induction therapy and rejection treatment

Routine prophylaxis is given for the most common and dangerous microbes during the first months after transplantation. Doses of the immunosuppressive agent are tapered to lower levels during the same time period.

There is also an elevated frequency of cancer in transplanted patients. The most common are local skin cancers as basilioma or squamous cell cancer, but the accumulated risk for cancer can be calculated to be four times to that of the general population (29).

### **1.3 CLINICAL ISLET TRANSPLANTATION**

#### **1.3.1 Indications for clinical islet transplantation**

As mentioned above immunosuppressive therapy is, at least today, compulsory for clinical islet transplantation. This medication constitutes the largest risk for the patient. The islet transplantation procedure as such is considered safe if patients are screened for bleeding abnormalities etc (30) and grafts with a high purity and low total volume are transplanted (31). However even in the most successful cases exogenous insulin is traded for immunosuppressive drugs. This limits the indications of the procedure since most patients with T1DM function well on conventional therapy.

There are three generally accepted situations where islet transplantation can be considered.

- The patient has already undergone a transplantation of another organ (usually kidney) and is therefore already on immunosuppressive medication. Here the patient can benefit from the islet transplantation without having to balance it with the added risk of starting immunosuppressive treatment. This category is most often referred to as “islet after kidney” (IAK) (32, 33).

- The patient has end stage renal disease and is planned for transplantation with a kidney from a deceased donor. Here one can add islets processed from the same donor, simultaneous islet kidney transplantation (SIK). Often islets from one or two additional donors are transplanted to potentiate the graft. The most common indication for pancreas transplantation is simultaneous pancreas kidney transplantation (SPK). The procedure is, however, limited to relatively young and physically fit patients receiving a pancreas from non-obese donor less than 55 years of age (34-36). Islet transplantation usually comes into question for patients that don't qualify for SPK or at centers/regions/countries that do not perform SPK (37).

- Islet transplantation alone (IA) has been the most common procedure during this century ([www.citregistry.org](http://www.citregistry.org)). The main indication is frequent hypoglycemia combined with an unawareness of symptoms of low blood sugar. Iatrogenic hypoglycemia is a major unresolved problem for many patients with T1D. It is the limiting factor in the management of T1D, causing some deaths as well as recurrent physical, and recurrent, or even persistent psychosocial morbidity (6).

### **1.3.2 Results obtained in clinical islet transplantation trials 2000-2009**

Coming into the new millennium insulin independence through islet transplantation was rare. During the last years of the decade 10-15 % of the treated patients were insulin independent at one year post transplant, however, up to 80 % still had function shown by measurable c-peptide (ITR Giessen [www.med.uni-giessen.de/itr](http://www.med.uni-giessen.de/itr)).

Clinical trials in islet transplantation have generally been small. There are no published prospective randomized clinical trials to date. The collaborative islet transplantation registry (CITR) ([www.citregistry.org](http://www.citregistry.org)) have collected data from 412 allo islet recipients transplanted 1999-2008 at 27 North American, 3 European and 2 Australian centers and publish annual reports.

In July 2000 the group in Edmonton, Canada reported (25) that they had achieved insulin independence in 7 consecutive cases with a follow-up of 4 to 15 months. Several things had been changed compared to previous protocols. First - All patients received islets due to severe problems with hypoglycemia (islet alone, IA). Second - A new steroid free immunosuppressive regime was used with daclizumab as induction therapy and sirolimus plus tacrolimus as maintenance. Third – Transplantations were repeated with islets from several donors (2-4) until the patient became insulin independent. Fourth – Islets were transplanted fresh. No period of culture preceded the transplantation. A mean of  $11,547 \pm 1604$  islet equivalents per kilo bodyweight (IEQ) was needed for the recipient to obtain insulin independence.

A study was initiated by the Immune Tolerance Network spreading the “Edmonton Protocol” to 9 centers on both sides of the Atlantic. Each center transplanted 3-5 patients. Here the results were more complex with varying results between centers. At one center all 4 transplanted patients were insulin independent at one year thereby fulfilling the primary endpoint. On the other hand there were three centers with a total of 11 transplanted patients where none did so. In total, 44% were insulin independent at one year and 58% had been so at least at one time point within the trial (38). A larger series of transplantations conducted in Edmonton showed that while >80% of the patients still had islet function at five years (showed as c- peptide production) and an absence of hypoglycemia, the median time until exogenous insulin was reinstated was 15 months and only 7.5 % were insulin independent at five years (39). Similar results are presented in the CITR report ([www.citregistry.org](http://www.citregistry.org)). Of the registered patients, 70% were considered insulin independent (at least 14 days without insulin) but only 39 % remained so after two years. 35% had lost all function three years after the last transplantation.

In 2004 The Minneapolis group presented a trial with 8 patients all becoming insulin independent after only one transplantation and one donor (mean  $7,271 \pm 1,035$  IEQ/kg) (40). Key features in the protocol included aggressive anticoagulant therapy surrounding the transplant, etanercept (a TNF  $\alpha$  inhibitor) and rabbit anti-thymocyte globulin induction. Five out of eight remained insulin independent after one year. There was a large discrepancy between the donors (101 kg BW/34 BMI) and recipients (60 kg BW/23 BMI), making the results of the single donor procedure difficult to interpret. The same group have later reported an insulin independence of 66% with a mean follow up of 3.4 years (41). This would indicate an improvement compared to the corresponding Edmonton figures of about 25% at three years. However the study includes only six patients and is too small to draw any firm conclusions from.

Rickels et al published a series of reports on the metabolic evaluation and performance of patients transplanted with islets (IA) (42, 43), clearly indicating an insufficient  $\beta$ -cell mass (22% of normal) even in insulin independent persons, suggesting that only a fraction of intraportally transplanted islets actually engraft in the liver.

Further follow-up on the Edmonton patients have shown a reduction of GFR and progression of albuminuria over 4 years of observation despite improved glycemia (44). The rate of decline in GFR was however extremely variable and difficult to predict. Tacrolimus or the combination of tacrolimus/sirolimus was primarily thought to be responsible for the findings, however further progression of diabetes nephropathy could not be ruled out. On the contrary, Warnock et al found no reduction of renal function after islet transplant compared to the general public or a group T1DM patients that received intensive medical therapy (45). This was done in a cross over study in 42

patients. The same study showed a reduction of 0.9% in HbA1c and less progression of retinopathy in the islet transplanted group.

The researchers in Edmonton have also published data showing that 31% of their recipients had developed de novo HLA antibodies after transplantation (46). 23% were still on immunosuppressive medication when the first antibodies occurred. In the patients that had discontinued this medication for various reasons (predominantly graft failure) 10/14 were broadly sensitized with a mean PRA of 89.5%. These antibodies will make it more difficult to find a suitable donor if the patient would be considered for a pancreas, islet or renal graft in the future. Cadradi et al (47) confirmed that all of their patients that had stopped immunosuppression had developed allo-antibodies. Considering development of HLA antibodies while on immunosuppression Ferrari-Lacaraz and colleagues did not find that they were more frequent than in kidney transplantation (48, 49).

Lehmann et al have shown superiority of small islets in graft survival (50, 51) and work on models for remodeling islets to a favorable size.

The studies above and others have in the last decade shown that insulin independence can be obtained by multiple islet infusions and in some cases by only one. Even in these successful cases, the engrafted  $\beta$  cell mass is only about a fourth of that of a healthy individual. Considering that the cut-off level where insulin has to be reinstated is in the region of 20%, only a slight reduction in  $\beta$  cell mass must occur before this line is crossed and the patient has to start taking insulin again. However the most common indication for islet transplantation during this decade has been recurrent severe hypoglycemia. To overcome these problems independence of exogenous insulin

is desirable but not needed. A lower degree of function from the islets is usually enough (39). In fact, more than 80% of the patients in the Edmonton series experience freedom from recurrent hypoglycemia for more than five years, but only 40% of the total in the CITR registry.

Few trials report on possible effects of islet transplantation on long term diabetic complications. However, small uncontrolled trials have reported positive effects on heart and vessels (52), kidneys (53, 54), eyes (55, 56) and peripheral nerves (57). Considering the number of transplantations performed in the last decade more data in this regard could soon be expected.

At its present state islet transplantation should be seen as a procedure in development, suitable only for carefully selected and well informed patients. The ITN trial showed the importance of experience and devotion at all levels. It can be easily argued that islet transplantation should only be performed at centers with a special interest in  $\beta$  cell replacement therapy and as often as possible within clinical trials. Prospective randomized trials are needed to firmly establish which patients that benefit most from the procedure and how to perform it most efficiently.

### **1.3.3 Practical issues in clinical islet transplantation today**

The pancreas from the deceased donor is harvested and transported in almost the same way regardless if the pancreas is intended for islet isolation or whole organ transplantation. The only difference is that the vessels are needed at the latter procedure. Criteria for accepting donors differ from center to center. They usually include age, weight, previous illnesses, circulatory stability, current medication etc. In the Nordic countries we accept all donated pancreases for isolation that come from non-

diabetic donors, of 25-70 years of age, where the kidneys are deemed suitable for renal transplantation. Since pancreas transplantation is an established procedure with superior clinical results, whole pancreas transplantation has priority.

Islet isolation is a demanding, labor intensive and expensive procedure. This has motivated the Nordic transplantation departments (Uppsala, Stockholm, Malmö, Göteborg, Oslo and Helsinki) (Figure 3) and others (58-60) to set up networks with a central islet isolation facility serving more than one islet transplantation unit. Pancreases are sent to the isolation lab for processing and islets are returned for transplantation. This also allows for easier exchange of islets suitable for specific patients across the network. However, most international islet transplantation groups still have their own isolation facility.

As in all other transplantations the so called cold ischemia time (CIT), the time between when blood supply is stopped in the donor and later is reinstated in the recipient, is critical with inferior results if it is prolonged (61, 62). In islet transplantation, however, it is the start of the isolation and not reinstated circulation that defines the endpoint for CIT. Attempts have been made in recent years to introduce new and innovative transport solutions to reduce the impact of transportation and CIT on islet isolation and transplantation outcome, thereby allowing longer CIT. Initial results have been promising (63, 64). This is further evaluated and discussed in this thesis (Paper I).

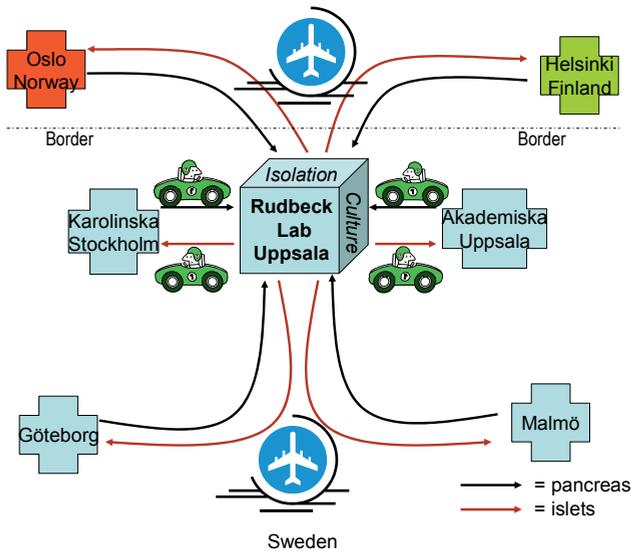


Figure 3. Configuration of The Nordic Network for Clinical Islet Transplantation. Six clinical sites share the same islet isolation facility. Pancreases and islets are transported by car or air depending on distance.

The isolation technique has only been modified slightly (65, 66) since Ricordi presented his automated method in 1988 (15).

Islet isolation can be divided into four steps.

1. The duodenum, excessive fat and connective tissue are removed from the graft and the pancreatic duct is identified.
2. Collagenase, responsible for disintegrating the tissue, is infused into the pancreatic duct.
3. The pancreas is put into a chamber at a specific temperature. In addition to the enzymatic process the chamber is shaken to physically separate the tissue. This incubation is stopped when samples taken from the chamber show free islets.

4. Exo- and endocrine tissue have different densities. This makes it possible to separate the tissue fragments from each other using a continuous gradient in a cell centrifuge (67).

Collagenase quality is of critical importance for the result of the isolation and may, unfortunately, vary between both producers and individual batches (68). This is one of the factors behind the variable outcomes of islet isolation. If optimal donor and transport criteria are fulfilled, an experienced islet isolation facility succeeds in producing islets suitable for clinical transplantation in ~50% of the isolations (38). It has been calculated that up to 80% of the original pancreas  $\beta$  cell mass can be successfully retrieved (69). Islets vary in size from only a few cells to half a millimeter in diameter. To standardize quantification of the graft a standard islet equivalent (IEQ), with a diameter of 0.15 mm has been used. Islet transplantation protocols often define that the graft size should be 5000 IEQ per kilogram body weight of the recipient or more to be transplanted.

Quality tests that are performed on the islet graft include viability tests, glucose stimulation tests and screening for microbes.

Following a successful isolation, islets can either be transplanted “fresh”, as in the classical Edmonton protocol, or be kept in culture awaiting transplantation. Clinical outcomes using the two approaches do not differ and today most groups prefer the later which has some substantial practical advantages. The time in culture allows for quality tests to be performed on the isolated islets, cross-matches made against possible recipients and finally travel to the transplant center and pretransplant preparation for the chosen patient. The transplantation hereby becomes a planned and most often daytime

procedure. Islet transplantation is normally performed within 72 hours after islet isolation.

Access to the portal vein is usually established either through a percutaneous transhepatic puncture guided by ultrasound or a small abdominal incision with cannulation of a mesenteric vein. Using the percutaneous route, the portal vein can be reached through a ventral puncture aiming at the left portal branch or lateral puncture aiming at the right branch. In our experience it's quicker and easier to get a good central placement of the catheter tip if the later technique is used. A problem with the lateral approach is however that breathing movements may dislocate the catheter. Islet transplantation using the transhepatic technique can be performed using local anaesthesia often combined with sedation. The patient is kept euglycemic through administration of iv insulin. Modern clinical protocols administer heparin (500-7000U) with the islet graft to avoid clotting and thrombosis. This increases the risk for bleeding from the puncture, when the catheter is removed. The use of a foam plug (30) at the exit site or delayed removal of the catheter, allowing coagulation parameters to normalize and local deposits of fibrin to counteract bleeding, has reduced this risk.

Islets have been shown to be vulnerable to glucotoxicity (70-72). Clinical results improve when patients have been kept euglycemic in the peri- and posttransplant period (73). Often tapering of insulin is not started until engraftment is expected to be established after about a month. This is in sharp contrast to pancreas transplantation which usually normalizes blood sugar levels already at the reestablishment of blood circulation.

Most immunosuppressive protocols in islet transplantation contain diabetogenic drugs, primarily tacrolimus ([www.citregistry.org](http://www.citregistry.org)). Balancing between efficacy and negative side effects calls for frequent contact with the patient and monitoring of the patients general condition, glucose levels and immunosuppressive drug levels. In the Nordic Network for Clinical Islet Transplantation the patient daily reports the last 24 h pre/post prandial and bedtime glucose levels together with insulin doses and hypoglycemia (if any) through telephone, fax or e-mail to the transplant center during the first month. After discharge from the hospital, trough levels of immunosuppression is measured twice a week together with c-peptide, creatinine and other lab tests. If no complications occur, outpatient visits are kept at once a week for the first three months following the transplant. Thereafter, visits and blood draws are spaced out in steps to once every three months. Decisions whether to repeat the transplantation are discussed and made together with the patient. Stabilization of blood sugar and normalization of HbA1c is most often achievable already after one transplant. If this was the goal for the transplantation and since we know that even when insulin independence is achieved it is usually lost within 2-5 years, it could be argued that a specific patient would benefit to accept a certain dose of exogenous insulin and only repeat islet transplantation when or if the initial positive result show signs of deterioration. In our experience, patients that have had severe problems with fluctuating blood sugars and hypoglycemia often cherish an improvement in this regard.

#### **1.3.4 Intraportal islet transplantation and alternative sites**

Islet infusion into the liver via the portal vein has been the “gold standard” for clinical islet transplantation. In animal models, several different islet transplantation strategies (i.e. deposition under the kidney capsule or intrasplenic) have functioned well, but in non human primates and humans intraportal islet transplantation is the only technique that repeatedly has lead to insulin independence. The efficacy is however low and little

is known for certainty about how the engraftment procedure takes place in humans. Until engraftment is fully established (74) islets have to rely on oxygen and nutrition to be delivered by the portal blood flow. This makes them vulnerable to a series of negative factors occurring during this period, i.e. high levels of immunosuppressive drugs in the portal vein (75, 76), hyperglycemia, IBMIR (see below) and low oxygen supply. In humans, islets do not seem to become incorporated in the liver parenchyma as in rodents, but rather stay in the portal vein lumen or wall (69). This, among other things, show that transplantation in rodents may be a poor model to understand engraftment in human islet transplantation.

Islet transplantation into the portal vein elicit a reaction called the instant blood mediated inflammatory reaction (IBMIR) (77-79). IBMIR is a, non-specific, reaction activating both inflammatory and coagulation pathways. It is induced by tissue factor (a transmembrane glycoprotein expressed on islets and i.e. within vessel walls). Tissue factor is down regulated during islet culture and this is one more reason that most groups today have abandoned the original Edmonton protocol to transplant islets immediately following islet isolation. Clinical trials trying to abrogate IBMIR through the selective blocking of coagulation and complement are presently ongoing.

The above described disadvantages concerning intraportal islet transplantation has stimulated investigations aiming to find sites better suited to harbor the graft. Striated muscle (80), omental pouch (81) and the native pancreas (82) are among the suggested sites, but all have yet to show equal or better efficiency than the intraportal route in humans.

Another implantation strategy and a way to evade the immune system would be to encapsulate the islets (83, 84) either in a chamber (macroencapsulation) (85) or spherical capsules where one or a few islets are enclosed in each (microencapsulation) (86, 87) . Semipermeable membranes allow small molecules to pass through (i.e. insulin and glucose) but keep the cells of the immune system out. The main advantage here is to be able to transplant without the need of immunosuppression and its side effects (1.2.2). Disadvantages with the macroencapsulated approach include fibroblastic overgrowth and placement. As islets are supplied via diffusion over the membrane, only thin layers of them inside the device can survive. Thus large surface areas are required, reducing the number of suitable implantation sites. Disadvantages with microencapsulation include incomplete encapsulation and gradual degradation of the material leading to a risk of sensitization. Clinical trials are few and no T1DM patients transplanted with encapsulated islets have become insulin independent. Clinical benefit has, however, been demonstrated by Calafiore et al with C-peptide production for several months. This was in a small study where recipients received islets placed in capsules of calcium alginate coated with polyornithine (86).

### **1.3.5 Monitoring the islet graft**

Clinical studies show that islet function is lost over time. Many theories have been presented on why this occurs. They include allo-rejection, toxicity of drugs and exhaustion because of too little islet mass from the beginning. Tools to monitor the islet graft are of vital importance to understand why, when and how function is decreasing. It would also allow treatment to be started if an unwanted development occurs, i.e. rejection. However methods to do this efficiently have been lacking. Metabolic evaluations have been adapted from endocrine research, e.g. insulin requirements, p-glucose, p-C-peptide, HbA1c, continuous subcutaneous glucose monitoring (CGMS)

and stimulation tests i.e. Mixed Meal Tolerance Test (MMTT), Glucagon Test, IV Glucose Tolerance Test (IVGTT) and glucose-potentiated arginine stimulation test. Some of these can be applied to calculate the functional beta cell mass, but it is usually too late to salvage function that has been lost between measurements and it gives no indication as to why the function has been influenced. More specific calculations for islet transplantation have been constructed (Beta Score (88), CGGCR/CPGR (89), SUIITO index (90)) but they invariably rely on the factors related to above and have no further predictive value. Islets can be found in needle biopsies from the liver, but only in about 30% of the cases and even when they are found they don't reflect overall graft function (91). A complicating feature is that transplantation usually has to be repeated for the patient to become insulin independent. This makes it difficult to evaluate each islet dose and to identify critical parameters in the donors, islet isolation processes, cultures, transports or transplantations and post transplantation patient management that are contributing to the final outcome. One possibility to evaluate specific factors contributing to the outcome in repeated transplantations is evaluated and discussed in this thesis (Paper V).

#### *1.3.5.1 Imaging Islet Transplantation*

Islet imaging is one of the fields where progress has been made in recent years. Ideally, a three dimensional description of the islets location should be made to see where changes have occurred and where ongoing inflammation/rejection is to be found. This could allow stereotactic directed biopsy and subsequent microscopic analysis. Imaging should also allow quantification of the functioning beta cell mass and consistently enable comparison both between individuals and between different time points in the same patient.

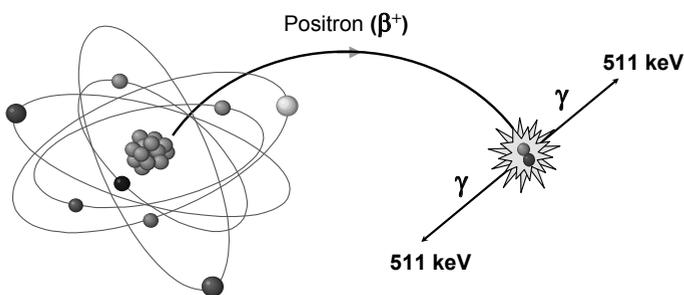
Clinically applied approaches involve positron emission tomography (PET) and magnetic resonance imaging (MRI). In both instances islets have been labeled in vitro, transplanted and subsequently followed by imaging in the recipient. This limits the interval when the graft can be monitored since the label will be cleared from the graft. Ideally, it should be able to inject an islet or beta cell specific probe and that way allow repeated imaging of the graft. To find such a probe has proved more difficult than originally thought (92).

Compared with radionuclide imaging, the main advantage of MRI is its capacity for high anatomic resolution. Islets have been labeled with superparamagnetic iron oxide particles (93) and in post transplant livers been depicted as (dark) signal voids in T2-weighted images up to six months. However no correlation was found when compared with the number of transplanted islets or clinical islet graft function. The total number of spots was generally low (maximum 138) and can be assumed to be aggregates of islets.

#### 1.3.5.1.1 Positron Emission Tomography (PET)

PET provides unique information about molecular and metabolic changes with disease. It has been used clinically the last 15 years (94) and is today an important tool in many aspects of clinical medicine. The most common area is oncology where PET with FDG (please see below) is used both for diagnostic purposes, mainly tumor staging, and treatment monitoring (95). PET has a high impact on the patient management and results in treatment strategy changes in approximately every third case, compared to if PET was not used (96).

Conventional radiological methods involve imaging of anatomy and morphology but by using PET various aspects of biological function can be imaged. A molecule, the function of which you want to image, is labeled with a radioisotope and the preparation is administered generally as an i.v. injection. The PET technique makes use of radioisotopes emitting positrons. In this decay, the positron immediately interacts with an electron and both are annihilated and converted to two, high energy (511keV), photons that travel 180 degrees from one and another (Figure 4). The photons are registered by the detector rings in the PET-camera as a “line of decay”. By analyzing millions of such lines the PET software constructs a PET image showing the location and concentration (Bq/cc) in the tissues of the administered PET tracer.



**Figure 4** The radioisotope emits a positron. The positron produced interacts with an electron. A reaction transforms the two particles into two photons of 511 keV emitted in exactly opposite directions.

Common radioactive isotopes are <sup>18</sup>Flourine (<sup>18</sup>F) and <sup>11</sup>Carbon (<sup>11</sup>C). The glucose analog Deoxy-Glucose linked to <sup>18</sup>Flourine constitutes Fluoro-Deoxy-Glucose (FDG) that is, by far, the most used PET tracer (>95% of all PET examinations). The radioactive half-life of FDG is 110 minutes. The cyclotrone where it is manufactured must therefore be situated in proximity (within 2 hours of transport) to the PET center where it will be used.

In conjunction with a standard PET examination a transmission investigation, generally using a  $^{68}\text{Ge}$  rod or point source, is performed to produce an “attenuation map”. This is used to correct the ensuing emission scan for attenuation. Using this information the original emission images are recalculated as if no attenuation had occurred of the photons.

The PET images show the relative location of the radioactive isotope, but the normal tissue accumulation of the PET tracer and the low spatial resolution of the technique makes it difficult to find anatomical landmarks to which the pathological PET findings may be related. To achieve an anatomical-morphological map, PET is therefore today generally combined with computed tomography (CT). Another synergistic advantage using a combined PET/CT hardware is that the attenuation correction needed for the PET can be derived from the CT data saving, at least, 25% of the time it takes to perform a conventional PET (97).

PET techniques in cell transplantation require the transplanted cells to be directly labeled with a radioisotope before transplantation. FDG enters the cell by using the same transporters as regular glucose and is phosphorylated into [ $^{18}\text{F}$ ]deoxyglucose-6-phosphate ([ $^{18}\text{F}$ ]FDG-6P) by hexokinase, but it cannot be further metabolized or leave the cell (98, 99), (Figure 5). The advantages of this modality are high sensitivity and immediate translation to clinical practice, since  $^{18}\text{F}$ -fluorodeoxyglucose ([ $^{18}\text{F}$ ]-FDG) is approved by the Food and Drug Administration (FDA) as clinical radiotracer (100). PET has been used to track transplanted cells like endothelial progenitor cells (101, 102) or cardiac stem cells (103) in different pre clinical settings (104).

FDG PET/CT as a method for monitoring the islet graft is evaluated and discussed in this thesis (Paper II-IV).

**In the Cell  $^{18}\text{F}$ -FDG is Phosphorylated to  $^{18}\text{F}$ -FDG-6-Phosphate**

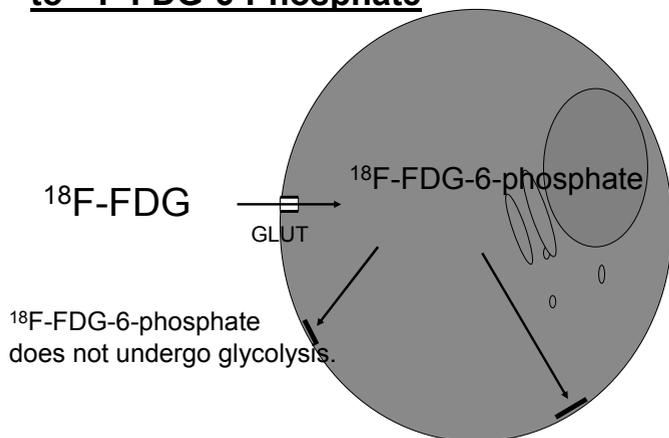


Figure 5.  $^{18}\text{F}$ -FDG enters the cell but is then trapped after phosphorylation.

## 2 AIMS

The general aim of this thesis was to study pre- and peri-transplant events that may influence the outcome of clinical islet transplantation.

The specific aims of the studies were:

- To evaluate the effect of a new method for continuous oxygenation of the procured pancreas.
- To establish and evaluate an imaging technique to study peri-transplant events in islet transplantation.
- To evaluate factors contributing to the outcome of repeated clinical islet transplantations.

## **3 MATERIALS AND METHODS**

### **3.1 ETHICS**

All studies were approved of in advance by either local or regional ethics boards regarding the clinical studies (Paper I, III, IV and V) and animal experiments (II) were approved by the local ethics committee for animal research in Uppsala. The studies were performed in accordance with national rules and regulations as well as international guidelines.

### **3.2 MATERIALS AND MODELS**

#### **3.2.1 Clinical pancreas procurement and transportation**

Organ procurement was performed within the Nordic Network for Clinical Islet Transplantation, which consists of six centers (Göteborg, Helsinki, Malmö, Oslo, Stockholm, Uppsala) and a central isolation unit located in Uppsala (60) (Figure 3). All pancreata were procured from brain-dead multiorgan donors. Pancreata were perfused in situ via the abdominal aorta with cold University of Wisconsin solution (UW) (ViaSpan, DuPont Pharmaceuticals Ltd., Herts, UK) or Histidine–tryptophan–ketoglutarate (HTK). CIT was defined as the interval between the aortic cross-clamp and initiation of the dissection procedure at the islet laboratory.

Pancreas shipment was performed utilizing a validated and certified organ transport system (Medco AS, Moss, Norway) that consisted of two plastic jars, four cooling aggregates, and an isolated transport container. For shipment, explanted pancreata were placed in the inner plastic jar and either immersed in 400 ml of cold UW, HTK or preserved by the Two Layer Method (TLM). In these cases explanted pancreata were placed on 200 ml of cold clinical-grade perfluorodecalin (PFD; Curavent, Pharmpur GmbH, Augsburg, Germany) that had initially been precharged for 30 – 45

min with 100% oxygen at a flow rate of 2000 ml/min. During oxygenation, the PFD was covered with a 100 ml-layer of UW. This procedure resulted in a partial oxygen tension of 750 – 800 mm Hg. Afterwards, the organs were fixed at the interface between the PFD and UW in a 600-ml container that was filled completely with UW and placed into the transport system for shipment.

### **3.2.2 Islet isolation**

#### *3.2.2.1 Clinical islet isolation and culture*

Isolation and culture procedures have previously been described in detail (65). Briefly, pancreata were dissected and perfused under low pressure ( $\leq 80$  mmHg) via the papilla Vateri with either Liberase HI (Roche, Indianapolis, IN) or Serva NB1 plus neutral protease (Serva, Heidelberg, Germany) dissolved in 80-120 mL cold Ringer's Acetate solution (RAS, Braun, Melsungen, Germany) with 3 mM  $\text{CaCl}_2$  (BDH Chemicals, Poole, UK) and 20 mM HEPES (Gibco-Invitrogen AB, Stockholm, Sweden). Perfusion volume was related to pancreas size.

Digestion was performed at 37°C using a continuous digestion filtration chamber filled with RAS supplemented with 10 mM nicotinamide, 0.5% penicillin-streptomycin, 0.4% sodium bicarbonate (Gibco), 20 mM HEPES, and 0.2  $\mu\text{l}/\text{mL}$  Pulmozyme (Roche Diagnostics Scandinavia, Bromma, Sweden). Harvested tissue was collected in RAS with 2% ABO-compatible human serum (Blood bank of the University Hospital, Uppsala, Sweden), centrifuged at 220 g, recombined and placed in 1.2-fold University of Wisconsin solution (Apoteket AB, Stockholm, Sweden) with 5  $\mu\text{l}/\text{ml}$  Pulmozyme for 1 hr incubation prior to purification.

Purification occurred on 1.077-1.097 g/mL linear Ficoll density gradients (Biochrom, Berlin, Germany) adjusted to 450-470 mOsm and 7.2-7.4 pH with 10x Hank's balanced salt solution (HBSS, Gibco), NaOH (1M) and 20 mM HEPES. Purified tissue was

collected in RAS supplemented with 2.5% ABO-compatible human serum and then washed twice with the same media prior to purity evaluation

For final culture, tissue fractions with similar purities were combined and resuspended in CMRL 1066 with addition of 10% ABO-compatible human serum, 10 mM HEPES, 10 mM nicotinamide, 2 mM L-glutamine, 50 µg/mL gentamycin, 0.25 µg/mL fungizone, 1 mM sodium pyruvate (Gibco) and 20 µg/mL ciprofloxacin (Bayer, Leverkusen, Germany). Tissue was cultured in platelet bags (Fenwal, Stockholm, Sweden) in a humidified atmosphere with 5% CO<sub>2</sub> at 37°C for 1 day and at 25°C thereafter. Islets were cultured for 1–5 days before transplant with medium change on day 1 and thereafter every second day.

#### *3.2.2.2 Porcine islet isolation and culture*

Pig islets were isolated from Swedish Landrace pigs using a standardized isolation protocol developed in Giessen, Germany (105). Islets were thereafter suspended in CMRL 1066, without bicarbonate (PAA Laboratories, Pasching, Germany); supplemented with 20% heatinactivated porcine serum and antibiotics 100 U/mL penicillin (Invitrogen AB, Sweden), 100 µg/mL streptomycin (Invitrogen AB, Sweden), and 20 µg/mL ciprofloxacin (Bayer Healthcare AG, Leverkusen, Germany); and cultured in culture bags (Baxter Medical AB, Eskilstuna, Sweden) at 37°C without CO<sub>2</sub> in a humidified atmosphere for 1–4 days.

#### **3.2.3 Islet quality controls**

Samples for the dynamic glucose-stimulated insulin secretion (GSIS) test were typically taken after 1 day of culture. A perfusion apparatus (Brandel, Gaithersburg, MD) was used to stimulate 20 hand-picked islets of about 150 µm diameter with low (1.67 mM) or high (16.67-20 mM) concentrations of glucose in Krebs's buffer. Timing of low and high glucose stimulation was as follows; low 36 min, high 42 min, and low

again for 48 min at a rate of about 1.5 ml/6 min. Samples were continuously collected but the first fraction used for analysis started at the 30 min time point and subsequent fractions changed every 6 min for a total of 16 samples in most cases. In 5 cases calculations for stimulation indices were adjusted due to fewer samples being taken.

Two methods of calculating the stimulation index were used. The standard method (Paper I, III, IV and V) discards transition values and uses only the average concentration of the middle six stimulated values divided by the average of the two initial and last three low glucose stimulated values:

$$\text{Standard Stimulation Index} = \frac{\text{Average of fractions 4-9 (54-84 min)}}{\text{Average of fractions 1-2 and 14-16 (30-36, 114-126 min)}}$$

The modified Uppsala Stimulation Index (Paper V) uses all values measured, taking into account the rate of change of released insulin in response to glucose concentration:

$$\text{Uppsala Stimulation Index} = \frac{\text{Average of fractions 3-9 (48-84 min)}}{\text{Average of fractions 1-2 and 10-16 (30-36, 90-126 min)}}$$

### **3.2.4 Labeling of islets**

#### *3.2.4.1 In vitro studies (Paper II and IV)*

For the porcine in vitro studies 300–900 islet equivalents (IEQs) were suspended in 100–200  $\mu\text{L}$  of Ringer's-acetate (30 mM; Braun, Melsungen, Germany)+10% human albumin 200 mg/mL (Baxter, Austria) and incubated with 5–20 MBq/mL of [ $^{18}\text{F}$ ]FDG for 60 min in 37°C (5% CO<sub>2</sub>) in a 50-mL Falcon tube (BD Biosciences, Erembodegem, Belgium).

For the human in vitro studies 1000 IEQ were suspended in 200  $\mu\text{L}$  Ringer Acetate + 10% human albumin and incubated with 20 MBq/mL [ $^{18}\text{F}$ ]FDG for 60 min at 37°C. Islets were also labeled with different concentrations of glucose in

the incubation media (2.7–22 mM).

#### *3.2.4.2 Porcine transplantation (Paper II)*

For the in vivo studies all islets, 90,000 –170,000 IEQs, were suspended in 5–12 mL of Ringer's-acetate+10% human albumin and incubated with 20 MBq/mL [<sup>18</sup>F]FDG for 60 min in 37°C (5% CO<sub>2</sub>). Finally, islets were washed three times with Ringer's acetate to remove all radioactivity not incorporated in the islets.

#### *3.2.4.3 Clinical transplantation (Paper III and IV)*

68 500–153 400 IEQ (15–30% of the administered islets) were suspended in 8–10 mL Ringer Acetate + 10% human albumin and incubated with 20 MBq/mL [18F]FDG for 60 min at 37°C. Islets were washed three times to remove extracellular radioactivity before transplantation.

### **3.2.5 Islet transplantation**

#### *3.2.5.1 Clinical islet transplantation (Paper I, III, IV and V)*

Islet recipients had previously received a kidney transplant. All transplantations, but one (Paper V), were performed through a percutaneous transhepatic cannulation of the portal vein. In the remaining case a mini laparotomy and cannulation of a mesenteric vein was needed to access the portal vein. Early on islets were infused by a syringe, but since the fall of 2003 islets have been infused by gravity from a transfusion bag. 500-5000 U of heparin was added to the islet bag or syringes before infusion. The patients' plasma glucose was kept stable at 4-8 mmol/L during transplantation by using iv insulin and frequent measurements.

### 3.2.5.2 *Porcine islet transplantation (Paper II)*

An upper abdominal midline incision was made on anesthetized pigs. A catheter (feeding tube, 0.9-mm inner Ø, Medicoplast, Illingen, Germany) was placed with its tip in the central aspect of the portal vein. At least 7000 IEQ/kg BW were suspended in transplantation medium containing Ringer's acetate, 10% human albumin, and 11 mM glucose and infused over the course of 20–30 min using a clinical feeding bag system (Baxter Medical AB, Eskilstuna, Sweden). Four pigs received 600 U of heparin (33–46 U/kgBW, Leo Pharma, Sweden) together with the islet infusion to mimic the clinical situation. The remaining six pigs did not receive any anticoagulant.

## 3.2.6 PET imaging

### 3.2.6.1 *Imaging porcine islet transplantation (Paper II)*

A GE Discovery ST scanner (PET/CT; General Electric) was used when available (n=5). Because this scanner is mainly reserved for clinical examinations, a Hamamatsu SHR 7700 PET scanner (Hamamatsu Photonics KK, Hamamatsu, Japan) was used at occasions (n=5). The PET/CT scanner is equipped with a 15.7-cm axial and 60-cm trans-axial field of view. A mean 96-min dynamic scanning sequence was acquired in 2D to include the liver and was followed by a 2D whole-body examination. A 5-min 3D examination of the whole liver was then performed. CT (140 kV, 80 mA) was used for attenuation correction and anatomical correlation of the PET findings. Whole-body intravenously contrast-enhanced (20 mL Omnipaque, 240 mg/mL) CT of the liver was performed after completion of the PET examination protocol. The Hamamatsu PET scanner uses an 11.4-cm axial and 14.3-cm transaxial field of view. A 60-min 2D dynamic emission scan was performed to include the liver, followed by a 5-min static examination of the head and bladder. Transmission scans (10–30 min) were acquired before each emission

scan using a rotating  $^{68}\text{Ge}$  point source. Images were reconstructed using a 4-mm Hanning filter.

### 3.2.6.2 *Imaging clinical islet transplantation (Paper III and IV)*

PET/CT examinations ( $n = 6$ ) were performed on a GE Discovery ST scanner (General Electric, Milwaukee, WI), with a 15.7 cm axial and 60 cm transaxial field of view. A 60-min dynamic scanning sequence (30 frames  $\times$  120 sec) was acquired in 2D mode to cover the liver and thereafter a static 10-min 3D acquisition of the liver was performed. This was followed by a 2D wholebody PET examination including the torso and head (4–5 beds, 4–6 min/bed).

Computed tomography (CT) was used for attenuation correction of the emission images and for anatomical correlation of the PET findings. The radiation dose modulation software (Smart mA) minimized the tube current of the CT scanner for each patient. The tube voltage was 140 kV and the tube current was regulated between 10 and 80 mA with the noise index set at 22 Hounsfield Units.

The PET images were reconstructed using iterative Ordered Subset Expectation Maximization algorithm, utilizing the standard software supplied with the scanner. The radioactivity concentrations in the PET images were recalculated to provide images of SUV by dividing the radioactivity concentration (Bq/mL) by the injected radioactivity (Bq) per body weight (g).

PET and CT images were analyzed on a Xeleris Work Station (General Electric, Milwaukee, WI). Results of the PET measurement are given as means  $\pm$  range.

### 3.2.7 Retention

Retention was defined as the time point where 50% of the original radioactive uptake had left the islets. Retention of  $^{18}\text{F}$ FDG into the porcine islets was determined by suspending 300 IEQs of  $^{18}\text{F}$ FDG-labeled islets ( $n=4$ ) in 500  $\mu\text{L}$  of

EDTA–porcine blood drawn from healthy nondiabetic pigs (normal plasma glucose range 5.4–7.5 mmol/L). At different time points, the tubes were centrifuged (160 g, 3 min), and the radioactivity in the supernatant and pellet was measured. All samples were analyzed in triplicate.

When analyzing human islets, 1000–2000 IEQ of [<sup>18</sup>F]FDG-labeled islets were suspended in 2 mL transplantation medium at room temperature. At defined time points (5, 15, 30, 60, 90 and 120 min) three samples were centrifuged (800 rpm for 1 min) and the radioactivity ratio between pellet and supernatant was measured.

### **3.2.8 Immunosuppression**

Porcine recipients (Paper II) received no immunosuppression.

Patients (Paper I, III, IV and V) received a modified Edmonton Protocol (25) consisting of daclizumab (1 mg/kg BW) given before transplantation and thereafter every other week to a total of five doses per transplantation, tacrolimus (trough level 5-10 ng/ml) and sirolimus (trough level 12–15 ng/mL the first 90 days and 7-10 ng/mL thereafter). The prednisone dose was tapered to 5mg per day pre-transplant and then stopped from the day of the first transplantation. Due to side effects related to sirolimus (mainly mouth ulcers) many patients were switched to mycophenolic acid (1000 mg BID) over time.

### **3.2.9 Clinical outcome**

Short term outcomes were analyzed in Papers I, III, IV and V. In the first paper islet function was assessed 2 weeks after transplantation, by calculating the C-peptide to glucose ratio (CP/G) (89). The CP/G was calculated as (fasting C-peptide (nmol/L)X100)/fasting glucose (mmol/L). This measure considers the basal insulin production as well as the corresponding glucose levels in the recipient. Plasma C-peptide values depend also on renal function as it is cleared through the kidneys. All

patients were previously kidney transplanted and had stable graft function, but between individuals function varied. In the following papers (III-V) adjustments were made for renal function in the recipients and C-peptide to the product of glucose and serum creatinine (CP/GCr) was used instead (89). Conventional units were also used instead of SI and assessments were at 4 weeks.

In Paper V repeated transplantations were analyzed. To assess the impact of each transplantation individually, the increment in CP/GCr was used,  $\Delta$  CP/GCr, subtracting the CP/GCr before transplantation from the one measured at 28 days post transplantation.

## 4 RESULTS AND DISCUSSION

### 4.1 PAPER I

Comparing TLM-preserved (n=103) and UWS-stored (n=97) pancreases there were no significant differences regarding donor BMI, gender nor pancreas weight. Neither did islet yield, calculated as IEQ/gram trimmed pancreas, differ between the groups. Decline in islet yield with prolonged CIT didn't reach statistical significance. However, a negative relationship between long CIT and SI was found in both groups. The percentage of pancreata that yielded the critical islet mass and quality needed for clinical transplantation was nearly identical in TLM- and UWS-stored organs when compared for 0 to <6 h (50 vs. 50%, NS), 6 to <12 h (43.5 vs. 45.8%, NS) and 12 to 18 h (19.0 vs. 12.5%, NS). However, when the transplantation rate was separately analyzed within each group, a significant drop was found after 12 to 18 h of TLM as well as UWS storage ( $P<0.05$  vs. 0 to <6 and vs. 6 to <12 h). This observation was paralleled by a decrease in SI after 12–18 h that reached significance in UWS ( $P<0.05$  vs. 0 to <6 h) but not in TLM-stored pancreata ( $P<0.06$  vs. 0 to <6 h). Overall, no difference in islet yield postculture was found between the groups.

When comparing the two cold storage solutions regarding elderly donors ( $\geq 60$  years) there were no differences in mean age, gender, BMI, CIT or pancreas weight. Islet yield tended to be higher, but not statistically significant, in the UWS-group. Neither was any significant differences found in islet purity, SI, islet recovery after culture or percentage of grafts transplanted.

A limited number of patients (12) transplanted with islets from only UW or TLM preserved pancreases were included in the posttransplant function analysis. No significant differences were observed between experimental groups in regard to posttransplant function after the first, second or third islet infusion.

Islet isolation is a demanding, labor intensive and expensive procedure. In the Nordic Network we have therefore chosen to have a central islet isolation facility. However, it can be difficult to keep Cold Ischemia Times (CIT) short of logistical reasons. Prolonged CIT, leads to inferior isolation (61, 106) and islet transplantation outcomes. The latter is shown in paper V in this thesis. It is therefore important to evaluate promising techniques that might allow longer CIT without damaging the pancreas. “The Two Layer Method” had previously in a series of publications been shown to result in superior results compared to cold storage in UWS (63, 107, 108). However, in our study (Paper I) no advantages with TLM compared to UWS in a clinical setting was found. The Edmonton group recently published a similar study and came to the same conclusions (109).

There may be several reasons for the discrepancy between previously published results and the now performed clinical studies. One is the factor that the oxygenation has been performed differently in the studies. While the smaller trials subjected pancreata to continuous oxygenation for a short period subsequent to cold storage, the large scale trials utilized oxygen-precharged PFD for the entire period of CIT.

Another important difference between the old smaller and the clinical trials is the duration of CIT. In the old trials the CIT was longer and the difference between the groups might not show unless CIT is above a certain length. In the present paper we divided the CIT into three groups, less than 6h, 6h to less than 12h and 12h to 18h. Surprisingly, no positive effects were shown with TLM in any of these groups. The percentage of islet isolations resulting in grafts of clinical grade (amount and quality of islets) was significantly lower in both experimental groups when CIT was 12h or more. Previous studies have suggested that TLM preservation would be especially useful in elderly donors (63). We could see no benefit in 61 donors (31 TLM/30

UWS) above 60 years of age, neither in islet yield, SI, purity or the number of isolations that led to clinical transplantation.

Factors such as tissue thickness, total size and histomorphological variables differ between the human pancreas and the different animal's pancreases that were previously used when evaluating TLM (110-112). Clearly the method functions well in a number of animal models, but that doesn't translate well to the human setting. On the contrary, it has been shown that only 20% of the human pancreatic volume is sufficiently oxygenated by TLM (113).

In Paper I, we have analyzed results of islet isolation from 200 pancreata. No beneficial effects were found when using TLM for cold storage. Neither were there any significant differences when comparing clinical outcome between groups. Since TLM doesn't offer any medical advantages and is both more logistically demanding and expensive than traditional UWS cold storage, it cannot be recommended in clinical islet transplantation. This is further emphasized by findings in Paper V of this thesis.

## **4.2 PAPER II, III AND IV**

In these three papers we have, in a stepwise approach, introduced the PET imaging technique in islet transplantation, first in a large animal model (Paper II) and thereafter in clinical transplantation (Paper III and IV). An initial a case study (Paper III), was followed by a pilot study including five patients (Paper IV).

In the porcine model (Paper II) 10 piglets were transplanted with an average of 430 000 IEQs (purity exceeding 95%). No differences were found in SI between FDG labeled and non-labeled islets. In vitro retention was 141 minutes. Autoradiography, ex vivo, of histological sections showed radioactivity in multifocal areas corresponding to the location of islets.

When transplanting FDG-labeled islets into the pig they were readily detected in the PET/CT images. Dynamic PET examinations showed a sharp rise in radioactivity in the liver during islet transplantation. However, the average peak was lower,  $54.0 \pm 5.1\%$  of administered activity, than expected. From the end of the infusion the radioactivity over the liver decreased with a  $t_{1/2}$  of  $216 \pm 16$  minutes. The AUC, measuring radioactivity in the liver (0-60 min), didn't differ significantly when comparing groups that had received heparin (n=4) or not (n=6).

Large fractions of the infused radioactivity were caught in “hot spots” in all transplantations. The localisation within the liver of these spots varied between pigs (Figure 6).

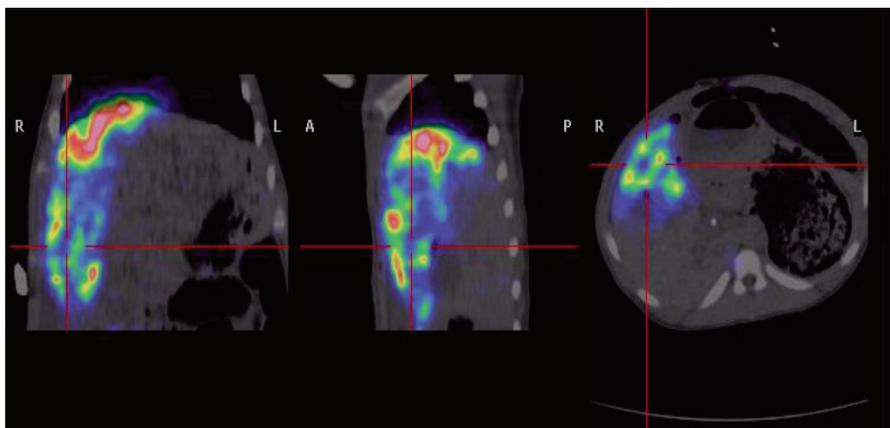


Figure 6. Radioactivity in the porcine liver 60 minutes after transplantation of FDG-labeled islets. The uptake is heterogeneously distributed. R=Right, L=Left, A=Anterior, P=Posterior. No radioactivity is seen in the lungs.

A whole-body examination was performed an hour after the transplantation. Apart from the liver and bladder, there was no specific accumulation of radioactivity in the body. When summarizing all uptake of radioactivity in the whole-body examinations 100%

of the infused radioactivity could be accounted for. Of special interest was that no accumulation was found in lungs, brain or heart (see below)

In this study, the use of PET/CT and FDG-labeled islets made it possible to dynamically image the peri-transplant phase of islet transplantation in a large animal model. At transplantation radioactivity was found only in the islets. These are generally too large to pass through the liver sinusoids. It was therefore expected that all infused radioactivity would be found in the liver. This was not the case. The maximum uptake in the liver at the end of the transplantation was only about 50% of the infused radioactivity. If islets were shunted past the liver they would, at least in part, be accumulated in the lungs, but no radioactive accumulation was found there. Even if an important reason to use FDG is that the radioactivity is “trapped” within the cell, dephosphorylation of [ $^{18}\text{F}$ ]FDG-6P to [ $^{18}\text{F}$ ]FDG could occur and this molecule would then be able to pass the cell membrane and leave the liver. If this was the case the uptake pattern of radioactivity would resemble that of [ $^{18}\text{F}$ ]FDG, with accumulation in brain and heart, but this was not seen. The fact that 100% of the infused radioactivity was accounted for, with about 50% accumulation in the liver and the remaining evenly distributed in the pig body or excreted in the urine, indicates that the extrahepatic radioactivity was represented by [ $^{18}\text{F}$ ]FDG-6P, which is not taken up by the CNS. Consequently, a probable interpretation of the present findings is that about 50% of the islet cells are damaged to an extent, within the first 20 min after the initiation of transplantation, that they release the entrapped [ $^{18}\text{F}$ ]FDG-6P, which leaves the islet cells and is thereafter rapidly and evenly distributed throughout the body of the pig.

Interpretation of these findings was initially cautious, since porcine islets are known to be more fragile than human. However, the findings fitted well with the observations made by Toso et al (114), where only 43% of the infused radioactivity was found in the rat liver an hour after intraportal islet transplantation. The mechanisms behind the possible damage to or destruction of islets can not be clarified by the presented technique. Little is known about the effects of hemodynamic stress with the high flow in the portal vein. We believe that the findings can be linked to the observations that islets, when encountering blood, elicit a strong innate inflammatory reaction, IBMIR (Instant Blood Mediated Inflammatory Reaction) (77, 79).

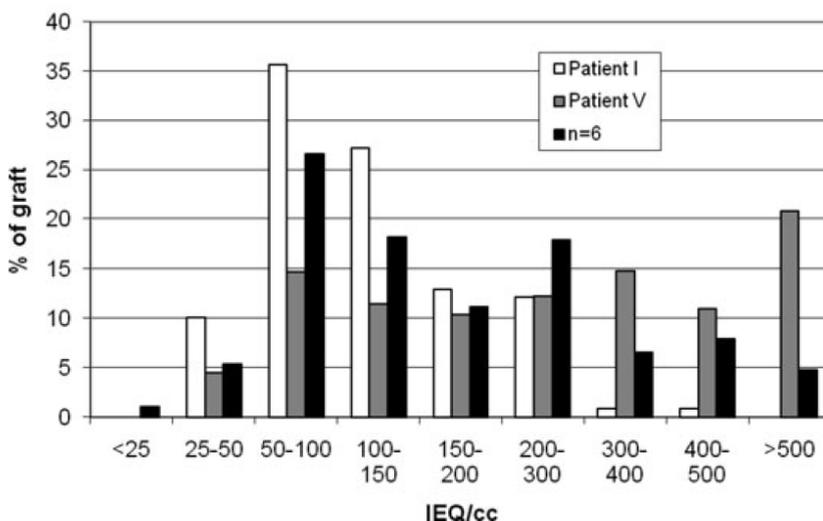
One of the main advantages attributed to the intraportal route of infusion of islets is that they would be distributed at a low density in a large and well-vascularized organ. The finding that islets are to a high degree concentrated in “hot spots” shows that this is not the case. There is no information regarding at what concentration islets can function or engraft within the liver. Supply of nutrients and oxygen could probably be hampered if they settle to close to each other.

Important conclusions from this porcine study were that FDG labeling of islets was feasible and that transplantation of the islets could be performed generating images of the initial transplantation phase. The PET/CT combination allowed quantification of initial engraftment and distribution in the liver.

When introducing the procedure in clinical transplantation five patients were transplanted with a total of 6 islet grafts. The first case was published in Paper III and also included in the five patients in paper IV. These will be discussed together. When adapting the procedure to human conditions only 23 % of the graft was labeled

instead of the whole graft. Retention half-life was 196 minutes in transplantation medium. Samples collected from the infusion bag just prior to transplantation showed that 89% of the radioactivity was still confined within the islets. The peak of radioactivity in the liver was found at 19 minutes and corresponded well to the finalization of the islet infusion. The decrease thereafter ( $t_{1/2}=79.6$  min (range 41.5-111.0)) was more pronounced than in the pig ( $t_{1/2}=216\pm 16$  min). As in the porcine experiments the distribution of radioactivity in the human liver was heterogeneous following transplantation. Large portions of the graft were found in multifocal areas in all segments of the liver. It was possible to present a “density profile” for each transplant showing how many percent of the graft that was found in areas with different levels of density (Figure 7).

### Islet density profiles of the graft



**Figure 7: The islet density profile presents the percentage of islets that have distributed in volumes with different IEQ/cc. Patient I, patient V and the average of all transplantations are shown.**

The variation between patients was wide. Volumes with concentrations of  $>400$  IEQ/cc liver tissue was 32% in the patient with the most pronounced “hot spots” and 1% of the graft in the patient with the most homogenous distribution. The importance of this finding is not known. In our material there was a tendency for better clinical results in the patients with a more homogeneous distribution. However the material is too small to draw any firm conclusions in this regard.

The mean maximum uptake of labeled islets in the human liver was 63% of the infused radioactivity. Even if 16% of the radioactivity may have left the graft in the form of free [ $^{18}\text{F}$ ]FDG (50–65 min between last wash to completed transplantation), only 75% of the radioactivity confined within the islets (63% out of remaining 84%) stayed in the liver. Poor islet quality and viability could theoretically explain these findings. However, the favourable clinical outcome and that no adverse effects on in vitro islet function were observed contradicts this notion. There was a rise of C-peptide ( $t_{1/2} = 25$  min), without physiological stimulus, which occurred during islet transplantation and continued for the first few hours after transplantation. The total amounts of C-peptide released can be calculated to 60–125 nmol, which corresponds to 10–21 U of insulin. The total insulin content in a pancreas varies substantially. In an investigation by Brandhorst et al., the mean insulin content in 20 pancreases was 116 U (115). Considering that not all islets from a pancreas are transplanted and that islets after transplantation are entrapped in clots causing a delayed release of C-peptide to the circulation, the calculated amount corresponds well with the assumption that 25% or more, of the islet graft is lost during the transplantation procedure.

### 4.3 PAPER V

To evaluate the clinical outcome of repeated islet transplantations we here introduced the  $\Delta$  CP/GCr ratio. This measurement utilizes c-peptide, and adjusts for renal function, glucose level and results already obtained by previous transplantations. Here we used this ratio to study pre-transplant factors and their possible influence on the short term results of 110, single donor, clinical islet transplantations.

The mean  $\Delta$  CP/GCr did not differ significantly between first (n=28), second (n=33), third (n=32) or fourth (n=17) transplants (p=0.502) (Figure 8).

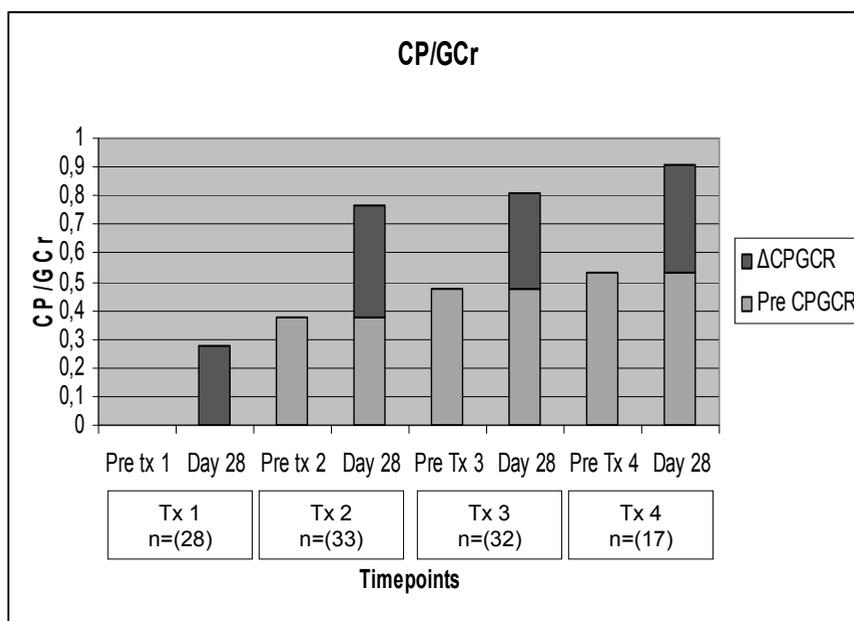


Figure 8:  $\Delta$ CP/GCr in relation to CP/GCr and order of islet transplantations. Mean CP/GCr at each time point (before and 28 days after each transplantation). At the first transplantation a number of patients received islets from more than one donor and that transplantation was therefore not included in this study. This is the main reason why there are more second and third transplantations than first. (Tx = transplantation).

The number of transplanted islets, the number of islets per kg of the recipient, islet quality (SI, USI) and the CIT all significantly related to the clinical short term outcome. No variables regarding the donor or the procurement operation reached statistical significance.

We had hypothesized that early function of kidney grafts from the islet donor could predict the outcome of the islet transplantation. If so, this would be important since islets are kept in culture for a couple of days after isolation. By monitoring early kidney function, transplantation of poor islet grafts would then be avoided. Each failed transplantation exposes the patient to an unnecessary procedural risk, intensified immunosuppression (30) and possible immunization (46). In the study we therefore collected creatinine and dialysis data from all recipients of kidneys from the islet donors and analyzed them in correlation to post transplant islet graft function. No correlation was found between the initial outcome of kidney transplantations and the islet transplantation. One possible explanation to this could be the multitude of other factors that influence the outcome of both procedures (i.e CIT, immunosuppression, islet isolation, surgical events etc) and may be different between the two organs on their way from procurement to engraftment in the recipient.

Results of this study indicate that the decision to use an islet preparation for clinical transplantation should be based on CIT in combination with data regarding the islet graft itself. Donor data, within our accepted limits, or the fate of organs transplanted from the same donor seem to be of less importance.

## 5 CONCLUSIONS

- Oxygenating the procured pancreas by utilizing the “two-layer method” does not improve isolation results or clinical outcome of islet transplantation.
- Porcine and human islets can be labeled with [<sup>18</sup>F]FDG and followed during and after islet transplantation by PET/CT. Radioactive half-life and retention of FDG limit observation time to 1-2 hours. The distribution of labeled islets in the liver is heterogeneous, with wide variations in islet density between different parts of the liver and patients. At least 25% of the [<sup>18</sup>F]FDG contained within the islets is lost during the transplantation indicating significant early damage to the graft.
- The number of islets, results of quality controls and the CIT are of importance for the short-term clinical outcome in islet transplantation.  $\Delta$  CP/GCr shows promise to become a useful tool in evaluating the effect of each graft in repeated islet transplantations. Early results of kidney transplantations from the islet donor do not predict the clinical outcome of the islet transplantation.

## 6 FUTURE PROSPECTS

Clinical islet transplantation still faces multiple challenges. Better preservation of the pancreas preceding islet isolation is needed to improve the outcomes. The present rate of about 50% of isolations leading to clinical transplantation must be improved. Interventions ensuring that more islets survive the transplantation itself and subsequent engraftment must prove successful. When these difficult tasks have been accomplished, the engrafted islet faces many of the same problems as whole pancreas grafts and other solid organ transplants.

A number of the issues above are high-lighted in this thesis. CIT is associated with inferior clinical outcomes. The use of TLM did not affect this relationship, perhaps because that too little of the gland could be oxygenated by this approach. However, logistical difficulties make it problematic to keep the CIT short. We are presently investigating a new, more lipophilic, substance and plan to evaluate it in a similar manner as TLM. As shown previously, and further emphasized in this thesis, islets are already damaged during the transplantation. IBMIR is an important part of this process. Methods to abrogate IBMIR have previously been identified. Our group is presently evaluating one of the most promising candidate drugs, Low-Molecular Dextran Sulfate (LMW-DS), in a clinical trial.

Islets are heterogeneously spread in the liver at the transplantation. Little is known about the significance of this finding, but it can be speculated that a high concentration of islets leads to poor oxygenation and limited nutrient supply. By characterizing more transplantations in this manner we hope to better understand this phenomenon. If it is found to be negative for the outcome, it may be possible to interact and achieve a more

homogeneous spread within the liver. The size of the Nordic Network for Clinical Islet Transplantation enables relatively large clinical studies to investigate new approaches.

The strength of PET/CT with FDG labeling in islet transplantation is that it can quantify islet distribution and initial survival. The technique is available at most institutions performing clinical islet transplantation today. To further investigate the fate of islets after transplantation, by PET, new tracers must be developed. These should be specific to  $\beta$ -cells or islets and allow repeated examinations to monitor the graft. Combinations of imaging techniques can also be used, ensuring that the strength of each modality is utilized.

$\Delta$  CP/GCr is introduced in this thesis. We plan to retrospectively investigate other factors that might be of importance for clinical outcome using this instrument. This ratio, for the evaluation of repeated islet transplantations, might also require further validation by others.

Several topics have been identified that need to be addressed to improve clinical outcome of islet transplantation. Ongoing research in all these areas should be able to promote further development in this field in the next few years.

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“Bok o vin”

The best wine-drinking, mountaineers and travel companions you can imagine.

“Båtgubbar”

Archipelago connoisseurs with a common past in Norra Latin.

Brothers, sister and brother-in-law with families

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## 8 REFERENCES

1. Mering J MO. Diabetes Mellitus nach Pancreasextirpation. Archiv für experimentellen Pathologie und Pharmakologie 1890;26:370.
2. Banting F BG. The Internal Secretion of the Pancreas. Journal of Laboratory and Clinical Medicine 1922:256.
3. Yalow RS, Berson SA. Immunoassay of plasma insulin in man. Diabetes 1961 Sep-Oct;10:339-44.
4. Hjern A, Soderstrom U. Parental country of birth is a major determinant of childhood type 1 diabetes in Sweden. Pediatr Diabetes 2008 Feb;9(1):35-9.
5. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. N Engl J Med 1993 Sep 30;329(14):977-86.
6. Cryer PE. Banting Lecture. Hypoglycemia: the limiting factor in the management of IDDM. Diabetes 1994 Nov;43(11):1378-89.
7. DuBois RS, Rodgerson DO, Martineau G, Shroter G, Giles G, Lilly J, et al. Orthotopic liver transplantation for Wilson's disease. Lancet 1971 Mar 13;1(7698):505-8.
8. Holmgren G, Ericzon BG, Groth CG, Steen L, Suhr O, Andersen O, et al. Clinical improvement and amyloid regression after liver transplantation in hereditary transthyretin amyloidosis. Lancet 1993 May 1;341(8853):1113-6.
9. Thomas ED, Storb R, Clift RA, Fefer A, Johnson L, Neiman PE, et al. Bone-marrow transplantation (second of two parts). N Engl J Med 1975 Apr 24;292(17):895-902.
10. Hering B, Jea. Islet Transplantation for Patients with Type 1 Diabetes. Graft 1999;2(1).
11. Lillehei RC, Simmons RL, Najarian JS, Weil R, Uchida H, Ruiz JO, et al. Pancreatico-duodenal allotransplantation: experimental and clinical experience. Ann Surg 1970 Sep;172(3):405-36.
12. Gruessner AC, Sutherland DE. Pancreas transplant outcomes for United States (US) and non-US cases as reported to the United Network for Organ Sharing (UNOS) and the International Pancreas Transplant Registry (IPTR) as of June 2004. Clin Transplant 2005 Aug;19(4):433-55.
13. White SA, Shaw JA, Sutherland DE. Pancreas transplantation. Lancet 2009 May 23;373(9677):1808-17.
14. Ballinger WF, Lacy PE. Transplantation of intact pancreatic islets in rats. Surgery 1972 Aug;72(2):175-86.
15. Ricordi C, Lacy PE, Finke EH, Olack BJ, Scharp DW. Automated method for isolation of human pancreatic islets. Diabetes 1988 Apr;37(4):413-20.
16. Scharp DW, Lacy PE, Santiago JV, McCullough CS, Weide LG, Falqui L, et al. Insulin independence after islet transplantation into type I diabetic patient. Diabetes 1990 Apr;39(4):515-8.
17. Warnock GL, Kneteman NM, Ryan EA, Rabinovitch A, Rajotte RV. Long-term follow-up after transplantation of insulin-producing pancreatic islets into patients with type 1 (insulin-dependent) diabetes mellitus. Diabetologia 1992 Jan;35(1):89-95.
18. Bosi E, Bottazzo GF, Secchi A, Pozza G, Shattock M, Saunders A, et al. Islet cell autoimmunity in type I diabetic patients after HLA-mismatched pancreas transplantation. Diabetes 1989 Jan;38 Suppl 1:82-4.
19. Braghi S, Bonifacio E, Secchi A, Di Carlo V, Pozza G, Bosi E. Modulation of humoral islet autoimmunity by pancreas allotransplantation influences allograft outcome in patients with type 1 diabetes. Diabetes 2000 Feb;49(2):218-24.
20. Tyden G, Reinholt FP, Sundkvist G, Bolinder J. Recurrence of autoimmune diabetes mellitus in recipients of cadaveric pancreatic grafts. N Engl J Med 1996 Sep 19;335(12):860-3.

21. Jaeger C, Brendel MD, Hering BJ, Eckhard M, Bretzel RG. Progressive islet graft failure occurs significantly earlier in autoantibody-positive than in autoantibody-negative IDDM recipients of intrahepatic islet allografts. *Diabetes* 1997 Nov;46(11):1907-10.
22. Huurman VA, Hilbrands R, Pinkse GG, Gillard P, Duinkerken G, van de Linde P, et al. Cellular islet autoimmunity associates with clinical outcome of islet cell transplantation. *PLoS One* 2008;3(6):e2435.
23. Fraenkel M, Ketzinel-Gilad M, Ariav Y, Pappo O, Karaca M, Castel J, et al. mTOR inhibition by rapamycin prevents beta-cell adaptation to hyperglycemia and exacerbates the metabolic state in type 2 diabetes. *Diabetes* 2008 Apr;57(4):945-57.
24. Zahr E, Molano RD, Pileggi A, Ichii H, Jose SS, Bocca N, et al. Rapamycin impairs in vivo proliferation of islet beta-cells. *Transplantation* 2007 Dec 27;84(12):1576-83.
25. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000 Jul 27;343(4):230-8.
26. Bumble V, Kamar N, Ribes D, Esposito L, Modesto A, Guitard J, et al. Long-term results in renal transplant patients with allograft dysfunction after switching from calcineurin inhibitors to sirolimus. *Nephrol Dial Transplant* 2005 Nov;20(11):2517-23.
27. Vincenti F, Friman S, Scheuermann E, Rostaing L, Jenssen T, Campistol JM, et al. Results of an international, randomized trial comparing glucose metabolism disorders and outcome with cyclosporine versus tacrolimus. *Am J Transplant* 2007 Jun;7(6):1506-14.
28. Fishman JA, Rubin RH. Infection in organ-transplant recipients. *N Engl J Med* 1998 Jun 11;338(24):1741-51.
29. Buell JF, Gross TG, Woodle ES. Malignancy after transplantation. *Transplantation* 2005 Oct 15;80(2 Suppl):S254-64.
30. Hafiz MM, Faradji RN, Froud T, Pileggi A, Baidal DA, Cure P, et al. Immunosuppression and procedure-related complications in 26 patients with type 1 diabetes mellitus receiving allogeneic islet cell transplantation. *Transplantation* 2005 Dec 27;80(12):1718-28.
31. Shapiro AM, Lakey JR, Rajotte RV, Warnock GL, Friedlich MS, Jewell LD, et al. Portal vein thrombosis after transplantation of partially purified pancreatic islets in a combined human liver/islet allograft. *Transplantation* 1995 Apr 15;59(7):1060-3.
32. Deng S, Markmann JF, Rickels M, Yeh H, Kim JI, Lian MM, et al. Islet alone versus islet after kidney transplantation: metabolic outcomes and islet graft survival. *Transplantation* 2009 Sep 27;88(6):820-5.
33. Toso C, Baertschiger R, Morel P, Bosco D, Armanet M, Wojtusciszyn A, et al. Sequential kidney/islet transplantation: efficacy and safety assessment of a steroid-free immunosuppression protocol. *Am J Transplant* 2006 May;6(5 Pt 1):1049-58.
34. Gruessner A, Gruessner R, Moudry-Munns K, Dunn D, Najarian JS, Sutherland DE. Influence of multiple factors (age, transplant number, recipient category, donor source) on outcome of pancreas transplantation at one institution. *Transplant Proc* 1993 Feb;25(1 Pt 2):1303-5.
35. Salvalaggio PR, Schnitzler MA, Abbott KC, Brennan DC, Irish W, Takemoto SK, et al. Patient and graft survival implications of simultaneous pancreas kidney transplantation from old donors. *Am J Transplant* 2007 Jun;7(6):1561-71.
36. Stratta RJ. Donor age, organ import, and cold ischemia: effect on early outcomes after simultaneous kidney-pancreas transplantation. *Transplant Proc* 1997 Dec;29(8):3291-2.
37. Lehmann R, Weber M, Berthold P, Zullig R, Pfammatter T, Moritz W, et al. Successful simultaneous islet-kidney transplantation using a steroid-free immunosuppression: two-year follow-up. *Am J Transplant* 2004 Jul;4(7):1117-23.
38. Shapiro AM, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, et al. International trial of the Edmonton protocol for islet transplantation. *N Engl J Med* 2006 Sep 28;355(13):1318-30.

39. Ryan EA, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, et al. Five-year follow-up after clinical islet transplantation. *Diabetes*2005 Jul;54(7):2060-9.
40. Hering BJ, Kandaswamy R, Ansite JD, Eckman PM, Nakano M, Sawada T, et al. Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. *JAMA*2005 Feb 16;293(7):830-5.
41. Bellin MD, Kandaswamy R, Parkey J, Zhang HJ, Liu B, Ihm SH, et al. Prolonged insulin independence after islet allotransplants in recipients with type 1 diabetes. *Am J Transplant*2008 Nov;8(11):2463-70.
42. Rickels MR, Schutta MH, Mueller R, Markmann JF, Barker CF, Naji A, et al. Islet cell hormonal responses to hypoglycemia after human islet transplantation for type 1 diabetes. *Diabetes*2005 Nov;54(11):3205-11.
43. Rickels MR, Schutta MH, Markmann JF, Barker CF, Naji A, Teff KL.  $\beta$ -Cell function following human islet transplantation for type 1 diabetes. *Diabetes*2005 Jan;54(1):100-6.
44. Senior PA, Zeman M, Paty BW, Ryan EA, Shapiro AM. Changes in renal function after clinical islet transplantation: four-year observational study. *Am J Transplant*2007 Jan;7(1):91-8.
45. Warnock GL, Thompson DM, Meloche RM, Shapiro RJ, Ao Z, Keown P, et al. A multi-year analysis of islet transplantation compared with intensive medical therapy on progression of complications in type 1 diabetes. *Transplantation*2008 Dec 27;86(12):1762-6.
46. Campbell PM, Senior PA, Salam A, Labranche K, Bigam DL, Kneteman NM, et al. High risk of sensitization after failed islet transplantation. *Am J Transplant*2007 Oct;7(10):2311-7.
47. Cardani R, Pileggi A, Ricordi C, Gomez C, Baidal DA, Ponte GG, et al. Allosensitization of islet allograft recipients. *Transplantation*2007 Dec 15;84(11):1413-27.
48. Ferrari-Lacraz S, Berney T, Morel P, Marangon N, Hadaya K, Demuylder-Mischler S, et al. Low risk of anti-human leukocyte antigen antibody sensitization after combined kidney and islet transplantation. *Transplantation*2008 Jul 27;86(2):357-9.
49. Hourmant M, Cesbron-Gautier A, Terasaki PI, Mizutani K, Moreau A, Meurette A, et al. Frequency and clinical implications of development of donor-specific and non-donor-specific HLA antibodies after kidney transplantation. *J Am Soc Nephrol*2005 Sep;16(9):2804-12.
50. Lehmann R, Spinass GA, Moritz W, Weber M. Has time come for new goals in human islet transplantation? *Am J Transplant*2008 Jun;8(6):1096-100.
51. Lehmann R, Zuellig RA, Kugelmeier P, Baenninger PB, Moritz W, Perren A, et al. Superiority of small islets in human islet transplantation. *Diabetes*2007 Mar;56(3):594-603.
52. Fiorina P, Gremizzi C, Maffi P, Caldara R, Tavano D, Monti L, et al. Islet transplantation is associated with an improvement of cardiovascular function in type 1 diabetic kidney transplant patients. *Diabetes Care*2005 Jun;28(6):1358-65.
53. Fiorina P, Venturini M, Folli F, Losio C, Maffi P, Placidi C, et al. Natural history of kidney graft survival, hypertrophy, and vascular function in end-stage renal disease type 1 diabetic kidney-transplanted patients: beneficial impact of pancreas and successful islet cotransplantation. *Diabetes Care*2005 Jun;28(6):1303-10.
54. Fiorina P, Folli F, Zerbini G, Maffi P, Gremizzi C, Di Carlo V, et al. Islet transplantation is associated with improvement of renal function among uremic patients with type 1 diabetes mellitus and kidney transplants. *J Am Soc Nephrol*2003 Aug;14(8):2150-8.
55. Venturini M, Fiorina P, Maffi P, Losio C, Vergani A, Secchi A, et al. Early increase of retinal arterial and venous blood flow velocities at color Doppler imaging in brittle type 1 diabetes after islet transplant alone. *Transplantation*2006 May 15;81(9):1274-7.
56. Lee TC, Barshes NR, O'Mahony CA, Nguyen L, Brunicardi FC, Ricordi C, et al. The effect of pancreatic islet transplantation on progression of diabetic retinopathy and neuropathy. *Transplant Proc*2005 Jun;37(5):2263-5.

57. Del Carro U, Fiorina P, Amadio S, De Toni Franceschini L, Petrelli A, Menini S, et al. Evaluation of polyneuropathy markers in type 1 diabetic kidney transplant patients and effects of islet transplantation: neurophysiological and skin biopsy longitudinal analysis. *Diabetes Care*2007 Dec;30(12):3063-9.
58. Benhamou PY, Oberholzer J, Toso C, Kessler L, Penfornis A, Bayle F, et al. Human islet transplantation network for the treatment of Type I diabetes: first data from the Swiss-French GRAGIL consortium (1999-2000). *Groupe de Recherche Rhin Rhjne Alpes Geneve pour la transplantation d'Ilots de Langerhans. Diabetologia*2001 Jul;44(7):859-64.
59. Goss JA, Schock AP, Brunicardi FC, Goodpastor SE, Garber AJ, Soltes G, et al. Achievement of insulin independence in three consecutive type-1 diabetic patients via pancreatic islet transplantation using islets isolated at a remote islet isolation center. *Transplantation*2002 Dec 27;74(12):1761-6.
60. Rydgard KJ, Song Z, Foss A, Ostraat O, Tufveson G, Wennberg L, et al. Procurement of human pancreases for islet isolation-the initiation of a Scandinavian collaborative network. *Transplant Proc*2001 Jun;33(4):2538.
61. Kaddis JS, Danobeitia JS, Niland JC, Stiller T, Fernandez LA. Multicenter Analysis of Novel and Established Variables Associated with Successful Human Islet Isolation Outcomes. *Am J Transplant* Jan 5.
62. Linn T, Schmitz J, Hauck-Schmalenberger I, Lai Y, Bretzel RG, Brandhorst H, et al. Ischaemia is linked to inflammation and induction of angiogenesis in pancreatic islets. *Clin Exp Immunol*2006 May;144(2):179-87.
63. Ricordi C, Fraker C, Szust J, Al-Abdullah I, Poggioli R, Kirlaw T, et al. Improved human islet isolation outcome from marginal donors following addition of oxygenated perfluorocarbon to the cold-storage solution. *Transplantation*2003 May 15;75(9):1524-7.
64. Tsujimura T, Kuroda Y, Kin T, Avila JG, Rajotte RV, Korbitt GS, et al. Human islet transplantation from pancreases with prolonged cold ischemia using additional preservation by the two-layer (UW solution/perfluorochemical) cold-storage method. *Transplantation*2002 Dec 27;74(12):1687-91.
65. Goto M, Eich TM, Felldin M, Foss A, Kallen R, Salmela K, et al. Refinement of the automated method for human islet isolation and presentation of a closed system for in vitro islet culture. *Transplantation*2004 Nov 15;78(9):1367-75.
66. Nano R, Clissi B, Melzi R, Calori G, Maffi P, Antonioli B, et al. Islet isolation for allotransplantation: variables associated with successful islet yield and graft function. *Diabetologia*2005 May;48(5):906-12.
67. Gray DW, McShane P, Grant A, Morris PJ. A method for isolation of islets of Langerhans from the human pancreas. *Diabetes*1984 Nov;33(11):1055-61.
68. Kin T, Johnson PR, Shapiro AM, Lakey JR. Factors influencing the collagenase digestion phase of human islet isolation. *Transplantation*2007 Jan 15;83(1):7-12.
69. Korsgren O, Lundgren T, Felldin M, Foss A, Isaksson B, Permert J, et al. Optimising islet engraftment is critical for successful clinical islet transplantation. *Diabetologia*2008 Feb;51(2):227-32.
70. Federici M, Hribal M, Perego L, Ranalli M, Caradonna Z, Perego C, et al. High glucose causes apoptosis in cultured human pancreatic islets of Langerhans: a potential role for regulation of specific Bcl family genes toward an apoptotic cell death program. *Diabetes*2001 Jun;50(6):1290-301.
71. Juang JH, Bonner-Weir S, Wu YJ, Weir GC. Beneficial influence of glycemic control upon the growth and function of transplanted islets. *Diabetes*1994 Nov;43(11):1334-9.
72. Biarnes M, Montolio M, Nacher V, Raurell M, Soler J, Montanya E. Beta-cell death and mass in syngeneically transplanted islets exposed to short- and long-term hyperglycemia. *Diabetes*2002 Jan;51(1):66-72.
73. Koh A, Senior P, Salam A, Kin T, Imes S, Dinyari P, et al. Insulin-heparin infusions peritransplant substantially improve single-donor clinical islet transplant success. *Transplantation* Feb 27;89(4):465-71.
74. Robertson RP. Islet transplantation as a treatment for diabetes - a work in progress. *N Engl J Med*2004 Feb 12;350(7):694-705.

75. Johnson JD, Ao Z, Ao P, Li H, Dai LJ, He Z, et al. Different effects of FK506, rapamycin, and mycophenolate mofetil on glucose-stimulated insulin release and apoptosis in human islets. *Cell Transplant*2009;18(8):833-45.
76. Shapiro AM, Geng Hao E, Lakey JR, Finegood DT, Rajotte RV, Kneteman NM. Defining optimal immunosuppression for islet transplantation based on reduced diabetogenicity in canine islet autografts. *Transplantation*2002 Dec 15;74(11):1522-8.
77. Johansson H, Lukinius A, Moberg L, Lundgren T, Berne C, Foss A, et al. Tissue factor produced by the endocrine cells of the islets of Langerhans is associated with a negative outcome of clinical islet transplantation. *Diabetes*2005 Jun;54(6):1755-62.
78. Moberg L, Johansson H, Lukinius A, Berne C, Foss A, Kallen R, et al. Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. *Lancet*2002 Dec 21-28;360(9350):2039-45.
79. Bennet W, Sundberg B, Groth CG, Brendel MD, Brandhorst D, Brandhorst H, et al. Incompatibility between human blood and isolated islets of Langerhans: a finding with implications for clinical intraportal islet transplantation? *Diabetes*1999 Oct;48(10):1907-14.
80. Rafael E, Tibell A, Ryden M, Lundgren T, Savendahl L, Borgstrom B, et al. Intramuscular autotransplantation of pancreatic islets in a 7-year-old child: a 2-year follow-up. *Am J Transplant*2008 Feb;8(2):458-62.
81. Berman DM, O'Neil JJ, Coffey LC, Chaffanjon PC, Kenyon NM, Ruiz P, Jr., et al. Long-term survival of nonhuman primate islets implanted in an omental pouch on a biodegradable scaffold. *Am J Transplant*2009 Jan;9(1):91-104.
82. Stagner JI, Rilo HL, White KK. The pancreas as an islet transplantation site. Confirmation in a syngeneic rodent and canine autotransplant model. *JOP*2007;8(5):628-36.
83. de Vos P, Marchetti P. Encapsulation of pancreatic islets for transplantation in diabetes: the untouchable islets. *Trends Mol Med*2002 Aug;8(8):363-6.
84. Scharp DW, Mason NS, Sparks RE. Islet immuno-isolation: the use of hybrid artificial organs to prevent islet tissue rejection. *World J Surg*1984 Apr;8(2):221-9.
85. Colton CK. Implantable biohybrid artificial organs. *Cell Transplant*1995 Jul-Aug;4(4):415-36.
86. Calafiore R, Basta G, Luca G, Lemmi A, Montanucci MP, Calabrese G, et al. Microencapsulated pancreatic islet allografts into nonimmunosuppressed patients with type 1 diabetes: first two cases. *Diabetes Care*2006 Jan;29(1):137-8.
87. Calafiore R, Basta G, Luca G, Lemmi A, Racanicchi L, Mancuso F, et al. Standard technical procedures for microencapsulation of human islets for graft into nonimmunosuppressed patients with type 1 diabetes mellitus. *Transplant Proc*2006 May;38(4):1156-7.
88. Ryan EA, Paty BW, Senior PA, Lakey JR, Bigam D, Shapiro AM. Beta-score: an assessment of beta-cell function after islet transplantation. *Diabetes Care*2005 Feb;28(2):343-7.
89. Faradji RN, Monroy K, Messinger S, Pileggi A, Froud T, Baidal DA, et al. Simple measures to monitor beta-cell mass and assess islet graft dysfunction. *Am J Transplant*2007 Feb;7(2):303-8.
90. Matsumoto S, Yamada Y, Okitsu T, Iwanaga Y, Noguchi H, Nagata H, et al. Simple evaluation of engraftment by secretory unit of islet transplant objects for living donor and cadaveric donor fresh or cultured islet transplantation. *Transplant Proc*2005 Oct;37(8):3435-7.
91. Toso C, Isse K, Demetris AJ, Dinyari P, Koh A, Imes S, et al. Histologic graft assessment after clinical islet transplantation. *Transplantation*2009 Dec 15;88(11):1286-93.
92. Sweet IR, Cook DL, Lernmark A, Greenbaum CJ, Wallen AR, Marcum ES, et al. Systematic screening of potential beta-cell imaging agents. *Biochem Biophys Res Commun*2004 Feb 20;314(4):976-83.

93. Toso C, Vallee JP, Morel P, Ris F, Demuylder-Mischler S, Lepetit-Coiffe M, et al. Clinical magnetic resonance imaging of pancreatic islet grafts after iron nanoparticle labeling. *Am J Transplant*2008 Mar;8(3):701-6.
94. Fletcher JW, Djulbegovic B, Soares HP, Siegel BA, Lowe VJ, Lyman GH, et al. Recommendations on the use of 18F-FDG PET in oncology. *J Nucl Med*2008 Mar;49(3):480-508.
95. Reske SN, Kotzerke J. FDG-PET for clinical use. Results of the 3rd German Interdisciplinary Consensus Conference, "Onko-PET III", 21 July and 19 September 2000. *Eur J Nucl Med*2001 Nov;28(11):1707-23.
96. Gambhir SS, Czernin J, Schwimmer J, Silverman DH, Coleman RE, Phelps ME. A tabulated summary of the FDG PET literature. *J Nucl Med*2001 May;42(5 Suppl):1S-93S.
97. von Schulthess GK, Steinert HC, Hany TF. Integrated PET/CT: current applications and future directions. *Radiology*2006 Feb;238(2):405-22.
98. Gallagher BM, Fowler JS, Guttererson NI, MacGregor RR, Wan CN, Wolf AP. Metabolic trapping as a principle of radiopharmaceutical design: some factors responsible for the biodistribution of [18F] 2-deoxy-2-fluoro-D-glucose. *J Nucl Med*1978 Oct;19(10):1154-61.
99. Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak CS, Pettigrew KD, et al. The [14C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. *J Neurochem*1977 May;28(5):897-916.
100. Howarth DM. The role of nuclear medicine in the detection of acute gastrointestinal bleeding. *Semin Nucl Med*2006 Apr;36(2):133-46.
101. Tamura M, Unno K, Yonezawa S, Hattori K, Nakashima E, Tsukada H, et al. In vivo trafficking of endothelial progenitor cells their possible involvement in the tumor neovascularization. *Life Sci*2004 Jun 18;75(5):575-84.
102. Aicher A, Brenner W, Zuhayra M, Badorff C, Massoudi S, Assmus B, et al. Assessment of the tissue distribution of transplanted human endothelial progenitor cells by radioactive labeling. *Circulation*2003 Apr 29;107(16):2134-9.
103. Terrovitis J, Lautamaki R, Bonios M, Fox J, Engles JM, Yu J, et al. Noninvasive quantification and optimization of acute cell retention by in vivo positron emission tomography after intramyocardial cardiac-derived stem cell delivery. *J Am Coll Cardiol*2009 Oct 20;54(17):1619-26.
104. Pearl J, Wu JC. Seeing is believing: tracking cells to determine the effects of cell transplantation. *Semin Thorac Cardiovasc Surg*2008 Summer;20(2):102-9.
105. Brandhorst H, Brandhorst D, Hering BJ, Bretzel RG. Significant progress in porcine islet mass isolation utilizing liberase HI for enzymatic low-temperature pancreas digestion. *Transplantation*1999 Aug 15;68(3):355-61.
106. Lakey JR, Warnock GL, Rajotte RV, Suarez-Alamazor ME, Ao Z, Shapiro AM, et al. Variables in organ donors that affect the recovery of human islets of Langerhans. *Transplantation*1996 Apr 15;61(7):1047-53.
107. Tsujimura T, Kuroda Y, Avila JG, Kin T, Churchill TA, Shapiro AM, et al. Resuscitation of the ischemically damaged human pancreas by the two-layer method prior to islet isolation. *Transplant Proc*2003 Nov;35(7):2461-2.
108. Tsujimura T, Kuroda Y, Churchill TA, Avila JG, Kin T, Shapiro AM, et al. Short-term storage of the ischemically damaged human pancreas by the two-layer method prior to islet isolation. *Cell Transplant*2004;13(1):67-73.
109. Kin T, Mirbolooki M, Salehi P, Tsukada M, O'Gorman D, Imes S, et al. Islet isolation and transplantation outcomes of pancreas preserved with University of Wisconsin solution versus two-layer method using preoxygenated perfluorocarbon. *Transplantation*2006 Nov 27;82(10):1286-90.
110. Avgoustiniatos ES, Hering BJ, Papas KK. The rat pancreas is not an appropriate model for testing the preservation of the human pancreas with the two-layer method. *Transplantation*2006 May 27;81(10):1471-2; author reply 2.
111. Deai T, Tanioka Y, Suzuki Y, Kuroda Y. The effect of the two-layer cold storage method on islet isolation from ischemically damaged pancreas. *Kobe J Med Sci*1999 Aug;45(3-4):191-9.

112. Tanaka T, Suzuki Y, Tanioka Y, Sakai T, Kakinoki K, Goto T, et al. Possibility of islet transplantation from a nonheartbeating donor pancreas resuscitated by the two-layer method. *Transplantation*2005 Sep 27;80(6):738-42.
113. Papas KK, Hering BJ, Guenther L, Rappel MJ, Colton CK, Avgoustiniatos ES. Pancreas oxygenation is limited during preservation with the two-layer method. *Transplant Proc*2005 Oct;37(8):3501-4.
114. Toso C, Zaidi H, Morel P, Armanet M, Andres A, Pernin N, et al. Positron-emission tomography imaging of early events after transplantation of islets of Langerhans. *Transplantation*2005 Feb 15;79(3):353-5.
115. Brandhorst HBD, Brendel MD et al. Assessment of intracellular insulin content during all steps of human islet isolation procedure. *Cell Transplantation*1998;7:489-95.

