Engraftment of Pancreatic Islets in Alternative Transplantation Sites and the Feasibility of \textit{in vivo} Monitoring of Native and Transplanted Beta-Cell Mass

DANIEL ESPES
Abstract

Islet transplantation is a possible curative treatment for type 1 diabetes (T1D). Currently the liver dominates as implantation site, despite the many challenges encountered at this site. Acute hypoxia in islets transplanted to muscle and omentum, two possible alternative sites, was prevailing. However, it was rapidly reversed at both implantation sites, in contrast to when islets were transplanted intraportally. At the intramuscular site hypoxia was further relieved by co-transplantation of an oxygen carrier, polymerized hemoglobin, which also improved the functional outcome. The complement system was activated after islet transplantation to muscle, but did not hamper graft function.

Both mouse and human islets transplanted to omentum become well re-vascularized and have a functional blood flow and oxygenation comparable with that of endogenous islets. Animals transplanted with islets to the omentum had a superior graft function compared with animals receiving intraportal islet grafts.

Alloxan-diabetic animals were cured with a low number of islets both when the islets were implanted in the omentum and muscle. The islet grafts responded adequately to both glucose and insulin and displayed a favorable mRNA gene expression profile.

A challenge in diabetes research and in islet transplantation is that there are no established techniques for quantifying beta-cell mass in vivo. By using radiolabeled Exendin-4, a GLP-1 receptor agonist, beta-cell mass after transplantation to muscle of mice was quantified. The results may well be translated to the clinical setting.

By comparing the pancreatic accumulation of $[^{11}\text{C}]$-5-hydroxy tryptophan ($[^{11}\text{C}]$-5-HTP) as detected by positron emission tomography (PET) in T1D patients with that of healthy controls, a 66% decrease was observed. This may in fact represent the loss of beta-cells, taking into account that other cells within the islets of Langerhans are largely unaffected in T1D.

In conclusion, the data presented support the use of alternative implantation sites for islet transplantation. In addition to improving the functional outcome this may enable more transplantations since the number of transplanted islets may be reduced. The techniques investigated for quantifying transplanted and endogenous beta-cell mass may greatly improve our knowledge of the pathophysiology of T1D and become a valuable tool for evaluation of beta-cell mass.

Keywords: Type 1 diabetes, Islet transplantation, Alternative implantation sites, Exendin-4, Positron Emission Tomography, 5-hydroxy tryptophan, Beta-cell mass

Daniel Espes, Department of Medical Cell Biology, Box 571, Uppsala University, SE-75123 Uppsala, Sweden.

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ISSN 1651-6206
ISBN 978-91-554-9551-0
urn:nbn:se:uu:diva-282953 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-282953)
“Sometimes the smallest things take up the most room in your heart”
Winnie the Pooh, A.A. Milne
List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.


III Espes D., Lau J., Quach M., Christoffersson G. and Carlsson PO. Restoration of Islet Vascularity and Oxygenation in Mouse and Human Islets Experimentally Transplanted to the Omentum: A Basis for Superior Function when Compared to Intraportally Transplanted Islets. *Submitted*

IV Espes D., Lau J., Franzén P., Quach M. and Carlsson PO. Function and Gene Expression of Islets Experimentally Transplanted to Muscle or Omentum. *Manuscript*

V Espes D., Selvaraju RK., Velikyan I., Krajcovic M., Carlsson PO. and Eriksson O. Quantification of Beta-Cell Mass in Intramuscular Islet Grafts using Radiolabeled Exendin-4. Accepted in *Transplantation Direct 2016*


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Abbreviations

AUC  Area under the curve
Bq   Becquerel
BHB  Bovine Hemoglobin
Bs-1 Bandeiraea simplifolica lectin 1
bp   Base pairs
CD31 Cluster of differentiation 31
CT   Computed Tomography
DAPI 4',6-diamidino-2-phenylindole
DCCT Diabetes Control and Complications Trial
DKA Diabetes ketoacidosis
EGFP Enhanced Green Fluorescent Protein
ELISA Enzyme-linked immunosorbent assay
GAPDH Glyceraldehyde 3-phosphate dehydrogenase
GCK Glucokinase
GFP  Green Fluorescent Protein
GLUT2 Glucose transporter 2
GPD2 Mitochondrial glycerol-phosphate dehydrogenase 2
HBSS Hanks Balanced Salt Solution
hESC Human embryonic stem cells
HIF-1α Hypoxia-Inducible Factor 1-alpha
HPRT Hypoxanthine guanine phosphoribosyl transferase
hPSC Human embryonic stem cells
IBMIR Instant blood-mediated inflammatory reaction
INS1 Insulin1
INS2 Insulin2
IVGTT Intravenous glucose tolerance test
ITT Insulin Tolerance Test
LADA Latent Autoimmune Diabetes in Adults
LDHA Lactate Dehydrogenase A
metHb Methemoglobin
MI Myocardial infarction
MMTT Mixed-meal tolerance test
MODY Maturity onset diabetes of the young
MRI Magnetic Resonance Imaging
NETs Neuroendocrine tumors
PB Phosphate Buffer
PCR: Polymerase Chain Reaction
PCX: Pyruvate Carboxylase
PDX1: Pancreatic and Duodenal homeobox gene 1
PECAM1: Platelet Endothelial Cell Adhesion Molecule
PET: Positron Emission Tomography
pO2: Partial pressure of O2
PolyHb: Polymerized hemoglobin
RBCs: Red Blood Cells
RPS7: Ribosomal Protein S7
SPECT: Single-Photon Emission Computed Tomography
T1D: Type 1 Diabetes
T2D: Type 2 Diabetes
TFF: Tangential Flow Filtration
TUJ-1: Neuron-specific class III beta-tubulin
VEGF-A: Vascular Endothelial Growth Factor A
qPCR: quantitative PCR
[11C]5-HTP: 11Carbon 5-Hydroxy tryptophan
[18F]-FDG: 2-Deoxy-2-[18F]Fluoro-D-glucose
Introduction

Homeostasis is the goal for all cells and organs. In the human body there are many physiological mechanisms present to maintain homeostasis. One of the most important components of homeostasis is the maintenance of normal blood glucose concentrations. The physiological mechanisms for glucose regulation must be adapted to several challenges including prolonged times of caloric restriction, excessive caloric intake, physical activity, sleep and many others which often occur on a daily basis. Many organs and cell types are involved in maintaining glucose homeostasis. However, there is only one cell type that secret a hormone which lowers blood glucose, i.e. the insulin producing beta-cell within in the islets of Langerhans. In type 1 diabetes the beta-cells are lost due to an autoimmune attack and thereby the ability to maintain normal blood glucose levels ceases. This thesis is focused on different ways to restore the beta-cell mass by transplanting the islets of Langerhans as a mean to cure type 1 diabetes and to establish imaging methods for the quantification of beta-cell mass.

The Pancreas

The pancreas is mainly an exocrine organ, in fact 98-99% of the cells are part of the organs exocrine function. But of outmost physiological importance (and crucial for this thesis) are the endocrine cells, i.e. the islets of Langerhans, which constitute the remaining 1-2% of the pancreatic volume. The pancreas in adults weighs 60-170 grams. Anatomically the gland can be divided in three regions, caput (head) with close proximity to the duodenum, corpus (body) and the caudal (tail) region. During embryogenesis the pancreas is formed from both the ventral and dorsal buds of the gut endoderm and therefore the different regions of the pancreas have separate blood supply (1). The caput region is supplied by the superior mesenteric artery whereas the corpus and caudal regions are supplied with blood from the coeliac artery. The pancreatic veins, containing blood rich in hormones, are exclusively drained to the portal vein. The pancreatic gland consists of several lobules divided by collagen. The smallest functional units of the exocrine pancreas is called acinus and consists of acinar-, centroacinar- and ductal cells. The exocrine pancreas has a crucial role for digestion and secretes many hormones/enzymes via the pancreatic duct into the duodenum.
The enzymes secreted can be divided into three main classes; lipases which digest fatty acids, proteases which digest proteins and amylases which digest carbohydrates. The enzymes are produced and stored as pro-enzymes in granules and activated when they reach the duodenum (2). In addition to the enzymes secreted, the pancreatic juice also contains mucins and a fluid rich in bicarbonate secreted from duct cells, which helps to neutralize the pH in the duodenum (3). In contrast to insulin, there are back-up mechanisms for the pancreatic enzymes since many of them are also produced and secreted, although in a much smaller amount, from the gastrointestinal tract (2). Despite that, dysfunction of the exocrine pancreas will have major implications for digestion and often lead to malabsorption.

There are a number of pathological conditions involving the exocrine pancreas, the most severe being pancreatic cancer. More common are acute pancreatitis which can lead to chronic pancreatitis. In turn this can lead to, apart from chronic abdominal pain, exocrine dysfunction. In addition, exocrine dysfunction is also common in patients with type 1 diabetes (T1D) (4), however the condition is often un- or misdiagnosed. In T1D, there is a reduction of the pancreatic volume of up to 50% (5-8), which is at least in part explained by the loss of insulin with its anabolic effects (9). Thus, the hormones secreted from the islets of Langerhans can also influence the function of the exocrine pancreas (9). In autopsy studies of pancreases from patients with recent onset T1D, signs of inflammation in the exocrine pancreas are often observed in addition to the loss of beta-cells (10, 11). If the exocrine dysfunction in T1D is part of the underlying pathology or a consequence of the beta-cell loss is, however, currently not fully understood (12). Although very intriguing, this lies beyond the scope of this thesis.

Islets of Langerhans

The islets of Langerhans are clusters of endocrine cells that are scattered throughout the pancreas. They were first described in the rabbit pancreas by the medical student Paul Langerhans in 1869, although their function was still unknown (13). However, in 1893 Edouard Laguesse “rediscovered” the islets in human pancreas and named them after Langerhans. Laguesse also proposed that the islets had an endocrine function involved in the control of blood glucose (14). There are at least five types of endocrine cells within the islets of Langerhans; beta-cells producing insulin, alpha-cells producing glucagon, delta-cells producing somatostatin, PP-cells producing pancreatic polypeptide and epsilon-cells producing ghrelin. In the islets of Langerhans in humans the beta-cells represent 50-80%, alpha-cells 15-20%, delta-cells 5-10%, and PP-cells and epsilon-cells approximately 1% of the endocrine cells. Apart from endocrine cells the islets of Langerhans also contain blood vessels and neurons (15).
Islet Vascularity, Blood Flow and Oxygen Consumption

The islets of Langerhans have a rich capillary network with a vascular density close to 10% (15). In fact, the glomerular-like vessels within the pancreas were observed and described in living rabbits already in 1882 before the function of the islet of Langerhans was known. In 1893, Laguesse proposed that the vascular network was important for secretion of something into the blood (9). In most islets (those with a diameter <150 µm) there is one afferent arteriole supplying the islet with oxygenized blood and nutrients, and a number of small efferent venules that drain the islets of blood rich in hormones. The efferent venules of these smaller islets are either connected to exocrine capillary plexa and form an insulo-acinar portal system or drain directly into larger veins. In larger islets (diameter >150 µm) there are one to three afferent arterioles entering the islet and a number of efferent venules that drain directly into larger veins which empty into the portal vein (16). In addition to the high vascular density, the islets also have a specialized endothelium which is ten-times more fenestrated than the endothelium in the exocrine pancreas (17). The fenestration is believed to facilitate the substantial hormone secretion to the blood stream (18).

The direction of blood flow within the islets has been a topic of debate. It has been proposed that the blood flow is directed from the core of the islet towards the mantle, known as B-A-D islet blood flow. Meaning that the blood would first reach the beta-cells and then blood containing insulin reaches the alpha-cells and finally the delta-cells (19). Most studies on this matter have been performed in rodent islets, but a B-A-D direction has also been proposed to be valid for human islets (20). However, an opposite model has also been proposed in which the blood flow is direct from the periphery (mantle) towards the center (core) (21, 22). In a recent publication with detailed studies of the islet blood flow pattern in pancreatic islets of mice it was described that, in fact, both patterns of blood flow are present (23).

The blood flow of pancreatic islets has been extensively studied in rodents using a number of techniques, reviewed by Jansson et al (24). Pioneering work in the 1980s by Claes Hellerström and Leif Jansson established the use of non-radioactive microspheres as a method for measuring islet blood flow in rodents (25, 26), and this is now considered to be the gold standard. Based on studies using the microsphere-technique in rats the islet blood flow has been found to be, when corrected for weight, 5-6 ml*min⁻¹*g islets⁻¹ which is one of the highest blood flow in the body in any organ. In addition, the islet blood flow significantly increases in response to glucose (26). The pancreatic islets thereby receive 10-15% of the total pancreatic blood flow despite that they only constitute 1-2% of the pancreatic volume (26).

The oxygen tension in islets was measured in vivo for the first time in 1998 by using modified Clark electrodes and was found to be 31-37 mmHg as compared with 20-23 mmHg in exocrine tissue (27). Interestingly, the
oxygen tension in endogenous islets was not affected by an intravenous bolus of glucose despite the increased metabolism in the beta-cells. The ability to maintain oxygen levels is probably explained by the high blood flow which increases even further in response to metabolic stimuli (26, 27). In fact, when the islet blood flow was decreased up to 50% by blocking NO synthesis the oxygen tension of the islets was unchanged whereas insulin secretion decreased (28). The importance of oxygen for the metabolism of glucose in beta-cells has been known since the 1960s. In an elegant study by Hellerström it was shown that glucose rapidly increases the respiratory rate and oxygen uptake of isolated islets (29). In perfused cultured islets it has been shown that the second-phase insulin secretion is reduced by 50% if the oxygen tension is reduced to <10 mmHg (30). In studies of mouse insulinoma βTC3 cells it has been demonstrated that lactate is produced, i.e. a sign of anaerobic metabolism, already at an oxygen tension of 25 mmHg but insulin secretion is not impaired unless the oxygen tension is < 7 mmHg (31).

Currently there are no available techniques for studying the blood flow of pancreatic islets in humans. However, there have been studies in which positron emission tomography (PET) has been proposed as a feasible technique for indirect studies of human islet blood flow (32, 33), and we are currently further elaborating this matter in Uppsala. Data regarding islet blood flow are at present restricted to studies in smaller animals including Atlantic hagfish, mice, rats and rabbits (24).

Diabetes Mellitus

The term diabetes mellitus includes all types of diseases with elevated blood glucose concentrations, i.e. hyperglycemia. Diabetes is Greek and means ‘to pass through’, whereas mellitus is Latin for ‘sweet’. The hallmark symptoms of diabetes are polyuria and polydipsia. Diabetes mellitus is divided into two main subclasses, type 1 diabetes (T1D) and type 2 diabetes (T2D). However, there are also other forms of diabetes, such as latent autoimmune diabetes in adults (LADA), maturity onset diabetes of the young (MODY), diabetes during pregnancy and diabetes secondary to other diseases. In 2011, the global prevalence of diabetes was estimated to 366 million, and the number is expected to reach 552 million by 2030 according the International Diabetes Federation (34). T1D is characterized by an almost complete loss of beta-cells due to an immune mediated attack, which causes hyperglycemia and hypoinsulinemia. T2D is characterized by insulin resistance and beta-cell dysfunction, often with increased serum insulin concentrations initially. However, there is a reduction in beta-cell mass over time also in T2D (35), and hence decreased insulin secretion.
Type 1 diabetes

T1D is a chronic disease which most often debuts during childhood or in adolescence. In patients with T1D the beta-cells are attacked and destroyed by the patient’s immune system (autoimmune disease) which eventually leads to a near complete loss of beta-cells and lack of endogenous insulin. In Sweden, T1D is one of the most common chronic diseases among children and young adults. Before the discovery of insulin in 1921 all patients suffering from T1D died either in an acute complication of the disease or from starvation. Since the introduction of insulin in clinical use there have, thanks to many technical and medical advances in the field, been dramatic improvements in the treatment of T1D. However, the life expectancy for patients with T1D is impaired despite modern treatment (36).

Modern insulin treatment is self-administered as subcutaneous injections of a long-acting insulin analog once or twice daily in combination with short-acting insulin analogs to every meal. Another option is the use of an insulin pump, which constantly delivers a short-acting insulin analog subcutaneously at a basal rate. A bolus dose, which is managed manually, is also administered through the pump to every meal. There are currently ongoing clinical trials of more sophisticated insulin pumps that not only deliver basal insulin at a constant prefixed rate but also measure the subcutaneous glucose concentrations and adjust the basal insulin dose accordingly, so called closed-loop insulin pumps (37).

However, it is almost impossible to mimic the normal physiology of blood glucose regulation. Some individuals with T1D even suffer from severe and rapid blood glucose fluctuations despite intensive self-monitoring of glucose concentrations and optimized insulin treatment, a condition known as ‘brittle diabetes’. These individuals are therefore living with a constant risk of life-threatening hypoglycemia. In those cases a pancreas or islet transplantation may dramatically improve the quality of life and even be life-saving (38).

Complications of Type 1 Diabetes

Patients with T1D may suffer from both acute and chronic complications due to the impaired metabolic control. The most common acute complications, which are potentially life-threatening, are hypoglycemia and diabetic ketoacidosis (DKA). The chronic complications of T1D can affect basically every organ of the human body. Chronic complications can be divided into microvascular (including retinopathy, nephropathy, neuropathy and foot ulcers) and macrovascular complications (including stroke, myocardial infarction (MI) and heart failure). The exact pathophysiological mechanisms are not fully understood but hyperglycemia play a central role for the development
of chronic complications. From the Diabetes Control and Complications Trial (DCCT) a causal link between glycemic control and the risk of complications has been established (39). Improved glycemic control by intensive insulin treatment can, however, prevent many of these complications (39). However, intensive insulin treatment also increases the risk for mild hypoglycemia as well as severe assisted-hypoglycemia and coma due to hypoglycemia (39, 40).

Hypoglycemia in T1D is caused by an imbalanced intake of exogenous insulin vs. carbohydrate intake which fails to be corrected by the counter regulatory hormones. Most patients with T1D experience mild hypoglycemia on a daily basis. However, after years of T1D some patients develop a phenomenon known as “unawareness”. In these patients the normal physiological response to hypoglycemia induced by stress hormones (adrenaline, noradrenaline and cortisone) such as heart palpitations and sweating are blunted and the affected patient is therefore not alarmed of the potentially lethal situation.

DKA accounts for half of all the deaths in young patients (<24 years of age) with T1D (41). DKA is caused by an absolute, or relative, insulin insufficiency, which increases lipolysis and thereby serum concentrations of free fatty acids. There is also a concomitant formation of ketone bodies in order to maintain energy supplies for the brain, which can only utilize glucose and ketone bodies for its energy consumption (42). However, the formation of ketone bodies decreases the pH level of the blood leading to the classic triad of DKA; ketonemia, acidosis and hyperglycemia (43). If not treated DKA is a lethal condition and even with modern intensive care unit treatment deaths do occur (43).

Retinopathy is often the first long-term complication to occur in patients with T1D. Already after seven years approximately 50% have retinopathy to some degree (44). The damage of small vessels in the retina is initially asymptomatic but will, if aggravated, lead to proliferative retinopathy with impaired vision and in worst case scenario blindness (44). In Sweden and many other countries, all patients with T1D are included in a screening program with examination of the retina every one to two years. Intensive insulin treatment is the best way to prevent retinopathy and to ameliorate progress once established (39, 40). Still, T1D is one of the most common causes for blindness among adults in the western world (45).

Nephropathy occurs in 30-50% of all patients with T1D and is a feared complication since it can lead to end-stage renal failure (46). In the DCCT study intensive insulin treatment proved to prevent the onset of nephropathy (39). However, intensive insulin therapy did not reduce the progression of nephropathy once microalbuminuria (30-300 mg/dl) was established (39). In fact, in a recent Cochrane meta-analysis of diabetic complications there were no studies that could prove a protective effect of intensive insulin treatment on the progression of nephropathy once established (40). However, by
treatment with angiotensin-converting-enzyme inhibitors the progression of nephropathy was ameliorated (47), even in the absence of hypertension (48). Diabetic nephropathy can eventually lead to end-stage renal failure which requires dialysis or kidney transplantation. T1D is one of the most common underlying causes for end-stage renal disease in the western world (46).

Neuropathy and ulcers are caused by poor microcirculation, most often in the lower extremities, which both damages nerves and impair wound healing. The ulcerations can be complicated by infections which may even affect the underlying bone, i.e. osteitis. Despite treatment with antibiotics, often multiple substances, the bacteria can be difficult to eradicate and there is a constant risk for bacteremia and sepsis. If the wound and infection are further aggravated, amputation is the only “curative” treatment option. In fact, diabetes is associated with 25-90% of all lower extremity amputations world-wide (49).

Ischemic stroke can cause a wide range of neurological symptoms depending on which artery, and thereby brain region, that is affected. In a follow-up report in 2005 based on the DCCT study it was shown that intensive insulin treatment reduces the risk of stroke by 57% (50). MI affects many people and is not specific for patients with T1D. However, patients with T1D have an increased risk of MI and an overall increased risk for cardiovascular disease which is at least 10-fold (51). In the initial DCCT study no significant risk reduction in cardiovascular disease was observed despite the intensive insulin treatment and improved glycemic control (39). However, in the extended follow-up study a reduction of overall cardiovascular disease of 42% was observed and reduction for non-fatal MI and death from cardiovascular disease was even greater (57%) (50).

In summary, T1D is associated with many severe complications which all greatly affect the life-quality and life-expectancy of those living with T1D. Improved glycemic control by intensive insulin treatment can reduce the risk of most complications but it is also associated with an increased risk of severe hypoglycemia (39). Obviously there is a need for further improvement in the treatment of T1D. Pancreas and islet transplantation can dramatically improve the metabolic control and reduce the risk of hypoglycemia. In fact an extensive systematic review of the clinical evidence for islet transplantation proved beneficial effects on long-term complications (52).

Pancreatic and Islet Transplantation

Since T1D is caused by a loss of beta-cells, the disease could potentially be cured by restoring beta-cell mass. Currently, there are in fact two possible means to do so. The first, and currently most successful strategy, is to transplant the whole pancreas from a brain-dead donor. This involves major surgery with potential risks and often a prolonged hospitalization in addition to
life-long immunosuppressive treatment. However, the patient survival rate is over 90% even after 3 years and around 80% of the patients have a functional graft (53, 54). There have even been reports on reversal of microalbuminuria and nephropathy lesions in patients receiving whole pancreas transplants which maintain normoglycemia for more than 5 years (55). In addition, systemic microvascular complications can be reversed within 12 months after simultaneous pancreas and kidney transplantation (56).

An alternative to whole pancreas transplantation is to transplant only the islets of Langerhans, which can be isolated from the pancreas of a brain-dead donor. Islet transplantation does not require major surgery, however, there is still a need for life-long immunosuppressive treatment. Based on the pioneering experimental work by Dr. Paul Lacy in the 1970s (57), clinical islet transplantation is predominantly performed as an infusion in the portal vein and the islets are thereby scattered throughout the liver. The first successful clinical islet transplantations were autologous and performed already in the 1980s (58, 59). In 1990 the first report on insulin independence (i.e. free from taking exogenous insulin) after allogeneic islet transplantation was published (60).

The procedure for islet transplantation is minimal-invasive and can be performed in local anesthesia and the patient is normally discharged from the hospital within a few days. However, up until the introduction of the Edmonton protocol in 2000 the results for islet transplantation were discouraging with approximately 10% of the patients maintaining insulin independence for one year. With the new steroid-free immunosuppressive regime and increased numbers of transplanted islets introduced in the Edmonton protocol, 80% of the patients remained insulin independent one year post-transplantation (61). Nevertheless, in the follow-up study it became obvious that the islet graft function deteriorated over time and after five years only 10-15% of the patients were still insulin independent (62). A more recent clinical study reported on improved long-term results with insulin independence five years post-transplantation close to 60% (63). However, in the later study a more aggressive immunosuppressive treatment regime was used as well as more islets, in many cases from three different donors (63). As for most organ transplantations there is currently a shortage of pancreases available for transplantation. Therefore, data on insulin independence are discouraging and especially when taken into account that two to three donor pancreases are needed in order to give a short term restored normoglycemia for one patient.

Many of the challenges in islet transplantation are related to the liver as implantation organ. First of all, when the islets are isolated they are disconnected from their extensive vascular network and unlike solid organ transplantations the vessels cannot be surgically anastomosed. Therefore, the islets are solely relying on diffusion of oxygen and nutrients for their survival until a new vascular network has been established. In the liver the oxygen
tension, even under normal physiological conditions, is only 5-10 mmHg as compared to the oxygen tension within the endogenous islets which is close to 40 mmHg (27, 64). Also, when the islets are infused into the blood stream an instant blood-mediated inflammatory reaction (IBMIR) occurs. This leads to activation of the complement and coagulation system causing a direct destruction of islets and formation of blood clots surrounding the islets which further aggravates the oxygen diffusion distance (65, 66). In a clinical case report, a portion of islets were pre-labelled with 2-Deoxy-2-[^18]F-fluoro-D-glucose ([^18]F-FDG) just prior to intraportal islet transplantation. The distribution of [^18]F-FDG (i.e. the islets) within the liver was monitored in vivo by PET and the islets were found to be evenly distributed throughout the liver. However, only 53% of the expected signal was detected which is suggestive of an acute loss of up to almost 50% of the islets (67).

In addition to the low oxygen tension in the liver, the revascularization of the islets in the liver is delayed (68-70). Islets transplanted to the liver have in experimental studies been shown to suffer from ischemia even one month post-transplantation (70). In experimental studies it has been shown that the islets transplanted to the liver become re-vascularized from the hepatic artery and that insulin secretion is only stimulated from the hepatic artery and not the portal vein (71, 72). The poor revascularization does not only hamper the delivery of oxygen and nutrients to the islets, but also the delivery of secreted hormones from the islets to the circulation. Also, the concentrations of immunosuppressive drugs, which have toxic effects on beta-cells, are higher in the portal blood due to the uptake from the gastrointestinal tract and the metabolism of the drugs in the liver (73). The glucose and lipid concentrations are higher in portal blood which further may contribute to the toxic effects on beta-cells (74). Furthermore, there have been reports on the formation of amyloid in islets transplanted to the liver (75-77).

In conclusion, the results for clinical intraportal islet transplantation are improving but they are still dependent on the use of multiple pancreases. Many of the challenges faced in islet transplantation are related to the liver as implantation organ. In order to improve the results of islet transplantation there is an increasing interest in the use of alternative implantation sites.
Alternative Implantation Sites for Islet Transplantation

When considering an alternative anatomical site for transplantation of islets there are a number of factors that need to be reconsidered, including physiological parameters, surgical safety, risk of harm to the normal organ function, immune privilege, potential for pre-conditioning and the potential to monitor the graft function and beta-cell mass post-transplantation.

In the experimental setting, many alternative implantation sites have been evaluated for islet transplantation including: pancreas (78, 79), muscle (80-84), omentum (85-91), beneath the kidney capsule (64, 92-94), spleen (93), subcutaneous tissue (95), gastric submucosa (96-98), testis (99), thymus (100), anterior chamber of the eye (101, 102), submandibular gland (103), and bone marrow (104). In the clinical setting autologous islet transplantation to muscle (80, 81, 105, 106) and bone marrow (107) has been evaluated. There is currently also an ongoing clinical trial in which allogeneic islet transplantation to the greater omentum is evaluated (P.I. Rodolfo Alejandro, Clinicaltrials.gov, identifier NCT02213003).

There are many physiological aspects that need to be considered in order to find an optimal anatomical site for islet transplantation. The site needs to have a high oxygen tension in order to match the oxygen needs of beta-cells.
In addition, this would potentially ameliorate the acute hypoxic phase post-transplantation during which the cells are depending on oxygen diffusion. The site should facilitate a rapid restoration of the glomerular-like vascular network of the islets as well as islet re-innervation. The physiological adaptation and modulation of the implantation site and the islets after transplantation are referred to as engraftment. The vascular network is important for the supply of oxygen and nutrients and thereby for the survival of the cells, but also for proper sensing of ambient blood glucose concentrations and secretion of hormones to the circulation. It has been shown that both the innervation and regulation of islet blood flow are important for the modulation of insulin secretion in response to increasing blood glucose concentrations (108). In this aspect it is interesting to note that muscle is one of the few organs which have a natural occurring angiogenesis (109). However, it has been postulated that the portal drainage of the pancreas serves of great importance since the liver is the main target organ for insulin action (110). Nevertheless, islet grafts transplanted to muscle have experimentally been proven superior compared to intraportal islet grafts despite the systemic venous drainage in muscle (80).

Apart from physiological parameters there are also surgical safety issues to consider. For instance, the pancreas is a tempting organ for islet transplantation from a physiological point-of-view. However, due to the possibility of leakage of exocrine pancreatic enzymes with concomitant risks of complications and mortality, the pancreas is presently not considered as a suitable site for clinical islet transplantation. In experimental studies, however, islets transplanted to the pancreas have been demonstrated to have an almost restored vascular network (111), and only moderate changes in their metabolic function (79). From a surgical safety point-of-view, the intraportal site has many advantages since the transplantation can be performed under local anesthesia in a minimal-invasive fashion and complications, such as portal thrombosis, are reported in less than 5% of the cases (112). Muscle is also a very attractive implantation site with regard to safety since it is easily accessible and there is vast experience from transplantation of parathyroid glands to the forearm muscle (113). In addition, in the clinical studies of islet transplantation to muscle there have been no reports of adverse surgical events (80, 81, 105, 106). In muscle, the islets can be surgically retrieved, even with a margin if needed, if any adverse event should occur. The greater omentum with its portal drainage is a promising candidate also in the aspect of surgical safety, even though the transplantation cannot be performed in local anesthesia. However, it can be performed as a laparoscopic operation and the omentum may be retrieved if needed.

Regarding the risk of harm to the implantation organ, most arguments must be considered as theoretical or speculative. There have been concerns raised regarding transplantation of a clinically relevant number of islets to the anterior chamber of the eye and the potential risk of impaired vision.
However, in large animal models no signs of impaired vision or other adverse events have been recorded (101). Also, the most widely used implantation site in preclinical studies, beneath the kidney capsule, may be considered as potentially harmful to the renal function of patients with T1D. One should remember that many of the patients that are eligible for islet transplantation already suffer from diabetic nephropathy, in addition to other complications.

There are a number of organs in the human body which are considered as immune privileged, including the anterior chamber of the eye, thymus and testis. All of these sites have been tested in experimental islet transplantation and the anterior chamber of the eye has even been investigated in non-human primates with promising results (101). However, there have also been reports indicating that the anterior chamber of the eye is not immune privileged and that transplanted islets in NOD-mice are destructed by immune cells as in the pancreas (114). Although, the search of an immune privileged site for islet transplantation is worth pursuing, since the possibility to transplant islets without, or even with less, immune suppression would make it an attractive treatment option for a much larger number of patients.

Since the acute hypoxia, which the islets are exposed to after transplantation, is a common challenge for all implantation sites it would be a great advantage if the implantation site could be pre-conditioned in order to stimulate angiogenesis and thereby diminish the oxygen diffusion distance. In that aspect muscle is a strong candidate, since it is easily accessible and has a naturally occurring angiogenesis (109).

There are many potential imaging techniques which may be applied to monitor beta-cell mass and function post-transplantation, including; PET, single photon emission tomography (SPECT) and magnetic resonance imaging (MRI). Although all these techniques lack the resolution required to monitor single islets, a composed islet graft may be detected. Therefore it is very challenging to monitor islets that have been transplanted to the liver, since they are scattered throughout the organ. In addition, many of the PET- and SPECT tracers in clinical use are metabolized in the liver, causing a high background signal. In intramuscular islet transplantation there are already reports on successful imaging of islet grafts with both MRI (80) and SPECT (105), although the number of beta-cells could not be quantified in these studies.

Biopsies are frequently used in solid organ transplantation in order to detect signs of rejection and to guide the immunosuppressive therapy. However, it is not relevant to harvest biopsies from the liver following islet transplantation, since the chance of finding islets is low. Therefore, it is currently not possible to predict rejection in islet grafts and to alter the immunosuppressive therapy in order to prevent it. In addition, it is not possible to discern immunological rejection from potential recurrence of disease after islet transplantation. If the islets were transplanted to muscle it would be possible
to acquire biopsies without risk for the patients’ health and in fact a smaller portion of the islets may be predesignated for biopsies and transplanted separately. Islets transplanted to the omentum would cause a greater challenge for imaging modalities due to its close location to other abdominal organs. Biopsies could be acquired from islet grafts in the omentum, but it would require a laparoscopic procedure and thereby making it far more invasive and not suitable for longitudinal follow-up. However, by transplanting islets to the anterior chamber of the eye it might even be possible to study the islets non-invasively \textit{in vivo} in a microscope and thereby monitor the survival, revascularization, signs of rejection and/or recurrence of disease (102).

In summary, there are many aspects that need to be considered before finding an optimal alternative implantation site for islet transplantation. Nevertheless, there are already many studies implying that the liver is not an optimal site for islet transplantation.

**Insulin Producing Cells Derived from Stem Cells**

Stem cells are a tempting and well elaborated source for insulin producing cells. Not only would a stem cell derived source of insulin producing cells solve the shortage of donated organs, but may also potentially make it possible to derive the cells from the recipient and thereby making the immune therapy obsolete. The clinical use of insulin producing cells derived from stem cells have, however, been an elusive dream for decades. Nevertheless, functional human insulin producing cells derived from stem cells are a reality today (115). In a study published already in 2005 human embryonic stem cells (hESCs) was differentiated into insulin positive cells, although not fully functional as beta-cells. The differentiation protocol included both \textit{in vitro} culture and \textit{in vivo} stimulation after co-transplantation with embryonic pancreas in mice (116). Also in a publication originating from the company ViaCyte (former known as Novocell) pancreatic endoderm cells derived from hESCs was differentiated into insulin producing cells with detectable insulin secretion when macroencapsulated and transplanted to mice (117). In a study published in 2012 from a separate group pancreatic progenitor cells were differentiated \textit{in vitro} from hESCs and then further differentiated \textit{in vivo} after transplantation into functional insulin producing cells with close resemblance to adult beta-cells (118). After engraftment \textit{in vivo} these cells secreted human C-peptide and responded functionally to glucose challenges (118). Using a modified protocol the same group has also evaluated the differentiation of pancreatic progenitor cells when transplanted in another macrocapsule and found that the cells survive and differentiate into insulin producing cells (119). In a more recent report from the same group (120) and another group (115) the entire protocol for differentiation from human pluripotent stem cells (hPSCs) to insulin producing cells was
adapted *in vitro* and thereby has the potential to produce a larger number of cells. In addition, the stem cell derived insulin producing cells were functional *in vitro*, cured diabetic mice after transplantation and responded to glucose challenges *in vivo* (115, 120). In a recent report using human insulin producing cells differentiated *in vitro*, clusters of cells were microencapsulated and transplanted to streptozotocin-diabetic mice which cured the mice and the grafts responded adequately to glucose challenges (121).

In order to take the next step using stem cell derived insulin producing cells, i.e. into clinical trials, there are however many concerns that needs to be addressed. First of all, and most importantly, there are safety issues regarding the use of stem cells concerning potential tumor development and other adverse events. There are also a number of ethical and legal perspectives regarding the use of stem cells since most of the cell lines used are derived from hESCs. Therefore the jurisdiction and political decisions will probably have a major impact on the use of all types of cells derived from stem cells in the clinical setting. Although of major importance, the ethical and legal perspective regarding the use of stem cells lies beyond the scope of this thesis.

Since stem cell derived insulin producing cells so far only have been used in animal models it is not known how the cells respond to the *in vivo* environment in humans. In this setting there will be several different stimuli from transcription factors, cytokines and hormones etc. In the *in vitro* setting these signals are tightly regulated and of major importance for the differentiation of the stem cells into the desired cell type (115, 116, 118). The potential of hESCs and hPSCs to form tumors/teratomas is a real threat and there have in fact been reports on the formation of teratomas in preclinical studies of stem cell derived insulin producing cells. When pancreatic endoderm cells derived from hESCs were macroencapsulated and transplanted to mice, seven out of totally 46 (15%) of the evaluated grafts contained teratomas (117). In a follow-up study from the same group they found that when using un-enriched pancreatic endoderm cells as origin the frequency of teratomas was even higher (46%) whereas if the cells were enriched based on the cellular marker CD142 prior to transplantation no teratomas were observed, although it should be noted that only seven grafts containing enriched cells were evaluated (122). In the study by Rezania *et al* formation of cartilage and bone was found in 50% of the transplanted animals (118). In the publication from Melton’s group, in which the insulin producing cells were completely differentiated *in vitro*, data are not provided regarding potential formation of tumors/teratomas after transplantation of the cells (115). In order to address the safety concerns regarding the use of stem cell derived insulin producing cells it therefore becomes apparent that the liver is not a suitable implantation site. Since the cells or clusters of cells would be widespread throughout the liver as in traditional islet transplantation they cannot be retrieved if there would be signs of tumor development. It would not even be possible to har-
vest representative biopsy material from the cells implanted in the liver. Neither are there any imaging techniques currently adaptable to cells transplanted to the liver as previously discussed. All in all, if stem cell derived insulin producing cells are transplanted to the liver the possibilities for monitoring would be very limited.

Regarding the choice of implantation site for insulin producing cells derived from stem cells, most arguments presented previously for islets holds true. However, the risk of tumor development adds another dimension of complexity since the cells should preferably be retrievable and easy to monitor. So far insulin producing cells derived from stem cells have either been transplanted beneath the kidney capsule (115, 116, 118), subcutaneously (macroencapsulated) (117, 119, 122) or the intraperitoneal cavity (microencapsulated cells) (121).

A possible route for using insulin producing cells derived from stem cells in the clinical setting is to encapsulate them prior to transplantation. This has already been tested for macroencapsulated pancreatic endoderm cells derived from hESCs with the aim to differentiate the cells further to insulin producing cells after transplantation (117, 122) and for microencapsulated insulin producing cells differentiated in vitro prior to transplantation (121). Thereby the cells are exposed to the in vivo factors (transcription factors, cytokines and hormones etc.) but could at any time point, at least when macroencapsulated, be retrieved and studied in detail regarding differentiation and potential tumor development. By using this approach it would be possible to gain valuable knowledge about how the cells respond to the in vivo environment in humans without, at least theoretically, great risks regarding the health and safety of the recipient. However, this requires a system for encapsulation that can meet the high physiological demands of insulin producing cells but still provide a sufficient barrier towards the recipient’s immune system and prevent cells to exit the capsule.

In summary, the use of insulin producing cells derived from stem cells has enormous potential since it may solve both the shortage of donated organs and, theoretically, also the hurdle of immunosuppressive therapy. However, the use of stem cells is entailed with many new challenges, especially the matter of safety regarding tumor formation.

Encapsulation of Islets and Insulin Producing Cells

An alternative to transplanting “naked” islets or insulin producing cells is to encapsulate them prior to transplantation. The underlying aim for encapsulation is to avoid immune suppressive treatment and all interactions with the recipients immune system, which thereby theoretically make it possible to perform xeno-transplantations. Encapsulation can be divided into two main categories, micro- and macroencapsulation. Microencapsulation means that
each individual islet or clusters of cells are encapsulated, usually in an alginate-based material. These encapsulated islets/cell clusters can then be transplanted to different implantation sites in a fashion similar to “standard” islet transplantation. In most experimental studies microencapsulated islets have been transplanted to the intraperitoneal cavity or beneath the kidney capsule. Macroencapsulation means that all the islets or clusters of cells are placed in one larger device which is surgically implanted, usually in the subcutaneous or intraperitoneal site.

Microencapsulation is not a new concept in islet transplantation, in fact the first experimental study was published already in 1980 (123). Since then there has been a number of studies including syngeneic- (124), allogenic- (125) and xeno-transplantation (126-129) of microencapsulated islets. There have even been clinical trials using microencapsulated islets with variable success (130-133). Microencapsulation has the advantage compared with macroencapsulation that the distance for oxygen delivery is shorter and the surface area is larger. Despite that, the long-term function of microencapsulated islets is poor which may, in addition to inadequate oxygenation, be explained by the formation of amyloid (134, 135). It should be noted that the formation of amyloid also occurs in islets transplanted to the liver (75-77) and potentially also in macroencapsulated islets. A disadvantage with microencapsulation is that it is close to impossible to assure the integrity and stability of each individual capsule. It is therefore a potential risk that immune reactions will occur due to leakage/breakage of capsules. In the worst case scenario this could also lead to seeding of tumorigenic cells from stem cell derived insulin producing cells.

Macroencapsulation has been studied in the field of islet transplantation since 1986 and in the first published study it was in fact fragments of human insulinomas that were encapsulated and proved to cure streptozotocin-diabetic rats (136). There have been many studies using macroencapsulated islets in animal trials (137-142), including xeno-transplantation (137, 142, 143) and even clinical trials of allogeneic islet transplantation (144, 145). One disadvantage with macroencapsulation is that the oxygen diffusion distance increases even further than in microencapsulated islets/cells and the surface area for oxygen delivery decreases. By incorporating an oxygen tank that can be filled with oxygen through external ports the diffusion distance for oxygen can be greatly reduced and sufficient oxygen delivery to the islets/cells ensured (140, 141, 145). The advantage with macroencapsulation compared with microencapsulation is that the integrity of the capsule/chamber can be tested and evaluated prior to transplantation. In addition, the chamber can easily be retrieved and the cells may be evaluated regarding function and in the case of stem cells also for differentiation and potential tumor formation. There is currently an ongoing clinical trial in which allogeneic islets are transplanted without immune suppression by using an oxygenized macrochamber produced by Beta-O2 technologies (PI
Per-Ola Carlsson, Clinicaltrials.gov identifier NCT02064309). Of interest, there is also an ongoing multicenter clinical trial evaluating the safety and efficacy of macroencapsulated insulin producing cells derived from stem cells (Viacyte, Clinicaltrials.gov identifier NCT02239354).

The great advantage with encapsulation is the barrier it forms towards the host immune system which thereby makes it possible to transplant cells without the use of immune suppression. However, while preventing large molecules, such as antibodies, to enter the capsule it has to be permeable for smaller molecules, such as nutrients and obviously hormones secreted by the encapsulated cells. Thereby the demands on the material used for encapsulation are high, since it needs to be selectively permeable. Another important and critical aspect of encapsulation is the delivery of oxygen to the encapsulated tissue. As mentioned before, endogenous islets have a very high oxygen tension (27) and the beta-cell function is impaired already at oxygen tension levels that are far above what is required for cell survival (30). In encapsulation revascularization is actively prevented and the transplanted islets/cells are therefore solely depending on oxygen diffusion for their entire life-time. The limiting distance for oxygen diffusion in tissue is 3-4 mm which becomes apparent in tumors since they will not grow larger than that without the formation of new vessels (146, 147). In the setting of encapsulation this could have detrimental effects on cell survival, function and in the case of insulin producing cells derived from stem cells also on their differentiation (148, 149).

Even if encapsulation of islets/cells may potentially solve the hurdle of immunosuppression there are many challenges. The choice of implantation site will probably have a major effect on the functional outcome for both micro- and macroencapsulated islets and in the case of stem cell derived material potentially also for the risk of tumor development.

**Positron Emission Tomography**

PET utilizes the special properties of certain unstable nuclides, which emit positrons when they decay. The positron is the positively charged antiparticle of the negatively charged electron. While the electron is highly abundant in our environment, the positron is exceedingly rare. This is due to the properties of antimatter, which states that when anti-particles encounter each other both will be annihilated. Consequently, an emitted positron will rapidly collide with an electron and the annihilation of both anti-particles will yield two anti-parallel high energy (511 kEv) photons which are emitted in 180 degrees to each other. The most commonly used positron emitting nuclides are Fluor-18 ($^{18}$F), Carbon-11 ($^{11}$C) and Gallium-68 ($^{68}$Ga), which have a half-life ($t_{1/2}$, meaning the time it takes until half of the present nuclides have decayed) of 109.8, 20.1 and 68.0 minutes respectively.
The PET-scanner consists of a ring of detectors, which registers the 511 keV photons emitted by the positron-electron annihilation events. A true annihilation event will yield simultaneous hits on opposite sides of the detector rings. Only these simultaneous hits (or “lines of response”) are counted during the scan in order to minimize the random noise caused by background radiation. By using advanced algorithms to reconstruct the sum of all registered “lines of responses” it is possible to “map” and localize the position of the emitted positrons in time and space within the PET scanner. However, the PET-examination does not provide any anatomical images and is therefore often combined with computed tomography (CT) and more recently also with MRI.

In PET, the tracer used is of essence for the application and interpretation of data. A tracer is a chemical or biochemical compound in which one stable nuclide has been replaced by a radioactive positron emitting nuclide. It is therefore important that the radioactive nuclides behave as their stable isotope, or that any change in molecular behavior is well characterized. By using different PET-tracers one can target and quantify different metabolic and biochemical processes \textit{in vivo}. Therefore, PET is often referred to as molecular or functional imaging. One of the most commonly used PET-tracers is $[^{18}\text{F}]$FDG which is a glucose molecule labeled with $^{18}$F. $[^{18}\text{F}]$FDG is transported into cells as glucose and phosphorylated by hexokinase into $[^{18}\text{F}]$FDG-6P but cannot be further metabolized and is therefore trapped within the cell. By using this PET-tracer it is possible to detect and image organs and tissues with a high glucose metabolism, which therefore makes it suitable for the detection of tumors.

The PET-examination itself can either be static or dynamic. In a static examination the PET-tracer is often administered prior to the examination and the subject is then scanned for a few minutes over the region in interest or the whole body. In a dynamic PET-examination the subject is first placed within the scanner and the PET-tracer is then administered while simultaneously starting the scanner. The positron decays are sorted by their “time-stamp” into different time frames for the duration of the examination. The resulting data set consists of several time-separated sequential 3D images of the tissues in the scanner, which makes it possible to study the kinetics of the PET-tracer in tissues of interest.

Apart from the specific uptake intended for the PET-tracer, certain organs may exhibit background uptake, which is not related to the desired enzymatic or receptor targeting properties. This is due to the metabolism and excretion of the tracer, which often occurs in the liver and/or kidneys.

Images from PET-examinations are often colorful and visually appealing. However, the great advantage with PET is the possibility of absolute quantification of the concentration of radiolabeled tracer in the living tissue. The counts detected by the PET-scanner, given that it is properly calibrated, can be converted into number of disintegrations per second (Becquerel (Bq)).
The concentration of Bq in any tissue (Bq/cc) can be determined by outlining the volume of interest from the CT or MRI images and thereby allows for comparisons between different scans within the same subject or between subjects. In addition, the uptake in specific organs can also be expressed as a standardized uptake value (SUV), which takes into account the total administered dose (Bq) of the tracer and the subject’s body weight (g) according to equation 1. Finally, Bq/cc can be converted into the number of molecules per cc (e.g. fmol/cc) in the target tissue, if the specific radioactivity of the compound is known (usually given as GBq/µmol).

\[
SUV = \frac{\text{Uptake}_{in\ tissue}(Bq) / \text{weight}_{tissue}(g)}{\text{Tracer\ administered\ (Bq) / Bodyweight(g)}}
\] (1)

**Imaging the Pancreas and Islets of Langerhans**

Imaging the pancreas as compared to the islets of Langerhans are completely different stories. Since the pancreas in most adults weigh 60-170 grams, it can easily be visualized by both CT and MRI, in fact even ultrasonography can be used to visualize and diagnose pathology of the pancreas.

It has been well established in studies using both CT and MRI, as well as in autopsy studies (7), that the volume of the pancreas is decreased in subjects with T1D (5, 6, 8). This can be explained, at least in part, by the loss of insulin secretion and its anabolic effects on the exocrine pancreas (9).

However, the islets of Langerhans cannot be distinguished even with high-resolution MRI. Imaging the endogenous islets of Langerhans is therefore a great challenge. In addition, they only constitute 1-2% of the pancreatic volume, while exhibiting no structural contrast towards the exocrine pancreas. Taking into account as well that the beta-cells only constitute 50-80% of the islet cells makes it an even greater challenge to image and quantify the beta-cell mass specifically. Considering the variation in pancreatic volume, islet percentage and beta-cell fraction, the expected variability in beta-cell mass even among healthy individuals may be as high as 8-fold. This is supported by autopsy studies in which the total endocrine mass among healthy individuals has been shown to have a 7-fold variation (35).

Islets have an average diameter of 150 µm, but most islets are much smaller with a diameter around 50 µm. However, there is a small number of islets with a diameter of about 300 µm, which contribute greatly to the total beta-cell mass (150). These largest islets could theoretically be detected with high-resolution CT or MRI since they have a spatial resolution of about 3-4 mm. Nevertheless, without a contrast agent it is difficult or close to impossible to delineate the endocrine cells from the exocrine pancreas.
The beta-cell mass depends on the size of the pancreas, the percentage of pancreatic islets and the beta-cell fraction within the islets. Due to the large variation even in healthy individuals the theoretical variation in beta-cell mass is as large as 8-fold. The image represents a schematic drawing of the pancreatic gland, a tile scan of a mouse pancreas stained for insulin (green) and finally a single mouse islet stained for insulin (green), glucagon (red) and nuclei in blue.

There have been several attempts to use MRI combined with contrast agents to image human pancreatic islets but none have so far been successful due to the limitations in resolution and specific signal from the islets. However, in preclinical studies of mice, MRI with manganese-chloride contrast has been used to visualize endogenous islets (151). The use of contrast agents could, however, be potentially harmful for the endocrine cells, since they must be administered in quite high doses (mmol range, as opposed to nmol for PET tracers). The same challenges also holds true for islets transplanted to the liver, since they are widespread. However, islets transplanted as clusters to muscle are possible to detect by MRI (80), but the technique does not allow for quantification of beta-cell mass.

By using PET, the limitation of resolution could be overcome by accurately measuring the sum of signals originating from the pancreas, i.e. the signal represent the total islet mass. Given that PET is combined with either CT or MRI for anatomical mapping the pancreas can easily be delineated, and thereby the pancreas specific uptake of the PET-tracer quantified. By such means, the accumulated tracer uptake from the islets, or even beta-cells, could be quantified. However, the accuracy of the technique depends exclusively on the specificity and kinetics of the PET-tracer.

Over the years a number of PET-tracers have been proposed to be markers of beta-cell mass, recently reviewed by Eriksson et al (152). For example $^{11}$C or $^{18}$F labeled dihydrotetrabenazine (DTBZ) have been extensively studied in the preclinical setting and was long considered a top candidate for imaging beta-cell mass (153). However, in a clinical study comparing healthy subjects to subjects with T1D the difference in the pancreatic uptake was only 13% (154).

SPECT shares similarities with PET in the use of radioactive nuclides and that it can be combined with CT. However, data obtained with SPECT are
based on the direct detection of gamma radiation from the nuclide compared with photon detection from positron-electron annihilation in PET. Due to this fact, the spatial resolution in clinical SPECT examinations is less than in PET-examinations, a potential major drawback when aiming to quantify beta-cell mass. Despite that, several studies have been conducted using SPECT with the aim to quantify beta-cell mass. In a recent study using SPECT and an $^{111}\text{In}$-labeled exendin tracer the pancreas could be visualized and the average pancreatic uptake was lower in subjects with T1D, although there was a substantial overlap with healthy subjects (155).

All in all, our current knowledge on human beta-cell mass relies on autopsy studies which, although they allow for absolute quantification, have major drawbacks. First and most obviously, it is not possible to monitor changes in beta-cell mass from autopsy material and second, it is difficult to determine which alterations that occurs in the pancreas just prior to death and post-mortem. One should keep in mind that this also holds true for our knowledge on beta-cell mass at the onset of T1D, which in most autopsy studies is found to be around 10-30% of that in healthy subjects (10, 156, 157). Although data and knowledge based on autopsy studies are of tremendous importance, it might not be representative for all patients with T1D. It has also been suggested that the beta-cell mass may fluctuate both in healthy individuals and during the development of T1D (158). However, without a non-invasive in vivo method for quantifying beta-cell mass these questions cannot be answered. The establishment of such a technique would therefore be of great importance for increasing our knowledge on the development of diabetes as well as a valuable tool for evaluating changes in beta-cell mass after islet transplantation.
Aims

General Aim
The overall aims of the work presented in this thesis were to evaluate alternative anatomical sites for pancreatic islet transplantation, to find means to improve the outcome of islet transplantation and to establish imaging methods for the quantification of beta-cell mass in the transplanted islets and in the native pancreas.

Paper I
This paper focused on evaluation of the degree of beta-cell hypoxia post-transplantation at the intramuscular site and whether co-transplantation of an oxygen carrier, polymerized hemoglobin, may improve the functional outcome.

Paper II
This paper focused on investigating the influence of complement activation and its importance for the functional outcome in intramuscular islet transplantation in an isogenic mouse model.

Paper III
This paper focused on evaluation of the engraftment of pancreatic islets transplanted to the greater omentum and whether the metabolic outcome is superior compared with intraportal islet transplantation.

Paper IV
This paper focused on examining the graft function following islet transplantation to muscle and the greater omentum. In addition, the genetic profile of the islet grafts is characterized and compared to that of endogenous islets.

Paper V
This paper focused on evaluating the feasibility of the PET-tracer DO3A-VS-Cys^{40}-Exendin-4 as a method for quantifying the transplanted beta-cell mass at the intramuscular site.
Paper VI
This paper focused on evaluating the feasibility of using the PET-tracer $[^{11}\text{C}]$5-hydroxy tryptophan ($[^{11}\text{C}]$5-HTP) as a surrogate marker for the native pancreatic endocrine mass \textit{in vivo}.
Materials and Methods

Animals

All experiments accounted for in this thesis were approved by the local animal ethical committee at Uppsala University. C57BL/6 and C57BL/6 nu/nu male mice weighing 20-30 grams were purchased from Taconic (Taconic M&B, Ejby, Denmark). In paper II, complement C3 deficient mice (C3 -/-) raised on a C57BL/6 background were used. The C3 -/- animals were raised by Dr. Marcela Pekna (Sahlgrenska Academy at University of Gothenburg) and shipped to Uppsala prior to the experiments. In paper III, C57BL/6 mice expressing enhanced green fluorescent protein (EGFP) under the Flk-1 (VEGFR2) promoter (i.e. in endothelial cells) or EGFP under the mouse insulin 1 promoter (i.e. in the beta-cells) were used in addition to C57BL/6 mice. The animals were housed under standardized conditions (12 hour light and dark cycle) in an animal facility with trained staff, who attended the animals daily. The animals had free access to food and water.

Prior to surgery, the animals were anesthetized with an intraperitoneal injection of Avertin (0.02 mL/g), a 2.5% solution of 10 g 97% 2.2.2-tribromoethanol (Sigma-Aldrich, St Louis, MO, USA) in 10 mL 2-methyl-2-buthanol (Kemila, Stockholm, Sweden), or sodium pentobarbital (200 mg/kg, Apoteket AB, Stockholm, Sweden) for terminal experiments. In the in vivo experiments when islet blood flow was investigated by direct microscopy, gas anesthesia with isoflurane (2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane) (Baxter Medical, Kista, Sweden) was used.

Isolation of Pancreatic Mouse Islets (Papers I - V)

Pancreatic islets were isolated from mice using a collagenase digestion and density gradient purification technique as previously described (69). Briefly, animals were anesthetized and the common bile duct was exposed by an incision in the skin and abdominal muscle. The liver lobes were displaced for better visualization of the common bile duct. A clamp was placed over the entrance of the bile duct into the duodenum. An ice cold collagenase solution (Clostridium histolyticum, 2.5 mg/ml, Roche Diagnostics, Mannheim, Germany) in Hanks balanced salt solution (HBSS) (National Bacteriological Laboratory, Stockholm, Sweden) was then injected through the bile
duct in order to fill the pancreas. The pancreas was surgically removed and placed in a 50 ml tube in a 37°C water bath for 18 minutes. Islets were separated from the exocrine pancreas by density gradient centrifugation using Histopaque-1077 and serum-free RPMI 1640-media (Sigma Aldrich). The islets were then handpicked and cultured free-floating in RPMI 1640 medium (Sigma-Aldrich) supplemented with L-glutamine (2 mmol/L; Sigma-Aldrich), benzylpenicillin (100 U/mL; Roche Diagnostics) and 10% (vol/vol) fetal calf serum overnight prior to transplantation. All islets were handpicked and counted prior to transplantation, islets with central necrosis or a damaged mantle were discarded.

Human Islet Isolation (Paper III)

All studies using isolated human pancreatic islets from brain-dead donors were approved by the regional ethical board of Uppsala. Islets from six heart-beating donors (age 54.7±5.2, n=4 male) were isolated at the human islet isolation core facility for the Nordic countries located at the Rudbeck laboratory (Uppsala University) as previously described (159). The insulin secretory capacity of the isolated islets was tested by glucose perifusion and expressed as a mean glucose stimulation index (7.1±1.4). The stimulation index was calculated as the ratio of the stimulated insulin secretion (20 mmol/l glucose) compared with the basal insulin secretion (1.67 mmol/l glucose) during islet perifusion. The human islets were cultured in CMRL1066 medium (Cellgro/MediaTech, Manassas, VA, USA), at a glucose concentration of 5.6 mmol/L, and with addition of 10% (vol/vol) bovine serum (Sigma Aldrich), L-glutamine (2 mmol/L; Sigma Aldrich), and benzylpenicillin (100 U/mL; Roche Diagnostics).

Synthesis of Polymerized Hemoglobin (Paper I)

Glutaraldehyde (70%), NaCl, NaOH, Na3S2O4, CaCl2-2H2O, sodium lactate, N-acetyl-L-cysteine, NaCNBH3 and NaBH4 were purchased from Sigma Aldrich. 100 kDa and 500 kDa hollow fiber cartridges were purchased from Spectrum Labs (Rancho Domniguez, CA, USA). All other chemicals were purchased from Fisher Scientific (Pittsburgh, PA, USA). Bovine hemoglobin (bHb) was purified from lysed bovine red blood cells (RBCs) by tangential flow filtration (TFF) (160).

Polymerization of bHb: Polymerized hemoglobin (PolyHb) was synthesized as previously described (161). The resultant PolyHb solution was clarified by filtering it through a column packed with autoclaved glass wool to remove large particles. The PolyHb solution was diluted from 1500 ml to 2000
ml, and then subjected to 4 cycles of diafiltration via TFF against an ice-cold modified lactate Ringer’s solution. The final concentration of PolyHb was 108.79 mg/ml with a P_{50} (pO_2 at which the bHb/PolyHb is half-saturated with O_2) of 2.66 mmHg, compared to 22.76 mm Hg for bHb. The metHb level of bHb/PolyHb solutions was measured via a cyanomethemoglobin method. The total protein concentration was measured according to the Bradford method using the Coomassie Plus protein assay kit (Pierce Biotechnology, Rockford, IL, USA).

O_2-bHb/PolyHb equilibrium curves were measured using a Hemox Analyzer (TCS Scientific Corp., Southampton, PA, USA) at 37°C. Samples were diluted with 5 ml of Hemox buffer (pH 7.4, TCS Scientific Corp.), 20 µl of Additive A, 10 µl of Additive B and 10 µl of antifoaming agent to a protein concentration of 60 µmol/l (heme), followed by equilibration with compressed air to a pO_2 of 145±2 mmHg at 37°C. Nitrogen was then used to deoxygenate the sample solution, while the bHb/PolyHb O_2 saturation (Y) was measured as a function of pO_2. The P_{50} and cooperativity coefficient (n) were regressed from curve fits of the experimental O_2-bHb/PolyHb equilibrium curves to the Hill equation using IGOR Pro (WaveMetrics Inc, Lake Oswego, OR, USA).

**Induction of Diabetes in Mice (Paper I - IV)**

Severe hyperglycemia was induced by a single intravenous injection of alloxan (75-90 mg/kg body weight, Sigma Aldrich) 3-5 days prior to transplantation. The higher dose of alloxan (90 mg/kg) was used in paper I for C57BL/6 nu/nu mice. Diabetes was defined as a blood glucose > 15 mmol/l on two consecutive days as measured by blood glucose reagent strips (Freestyle Lite, Abbot, Alameda, CA, USA). The effects of alloxan on the islet of Langerhans was initially described in the 1940s when researchers observed that rabbits injected with the substance developed hyperglycemia and many died (administered doses of 200-300 mg/kg). Histological evaluation of the pancreas revealed a selective necrosis of the beta-cells (162). Later it was found that the toxic effects of alloxan impairs the oxidative metabolism and there is no effect on the cellular membrane (163). More recently it has been described that the beta-cell specific effects of alloxan can be explained by its uptake through low-affinity GLUT2 glucose transporters, which are highly expressed in beta-cells, and generate reactive oxygen species and thereby necrosis of the cell (164). When administered in high doses, alloxan can also cause damage to hepatocytes and renal tubular cells, which express GLUT2. Nevertheless, in alloxan-diabetic animals the kidneys have a normal appearance and liver damage is rarely observed (165).
Experimental Islet Transplantation

Intramuscular Islet transplantation (Paper I, II, IV and V)
The abdominal muscle was exposed by a midline incision in the skin. The islets were collected in the center of a petri dish under inspection in a stereo microscope and then aspirated in a volume of approximately 50 µl medium with a butterfly-needle (25G) connected to a syringe prefilled with 200 µl HBSS. The butterfly-needle was inserted into the abdominal muscle in line with the muscle fibers under visual inspection in a microscope and the islets were injected in a total volume of approximately 100 µl. When co-transplanting islets with PolyHb as in paper I, the syringe was prefilled with media containing PolyHb instead of HBSS. The skin was then closed with sutures.

Islet transplantation to the greater omentum (Papers III and IV)
The stomach was exposed by a midline incision in the skin and abdominal muscle, and then fixated by a humidified surgical drape. The greater omentum was identified as the part connected to the greater curvature of the stomach. By carefully grabbing the omental fat the omentum was slightly stretched and a loose ligature placed around it. At a distance from the ligature an opening between the sheets of omentum was carefully made with a cannula. At all points the tissue was kept humid by carefully dripping saline on it. The islets were packed in a braking pipette under visual inspection in a stereo microscope. By entering the opening made by the cannula the islets were then infused into a pouch of the greater omentum, which was closed with the ligature. The abdominal muscle and skin were closed with sutures.

Intraportal Islet Transplantation (Paper III)
The portal vein was exposed by a midline incision in the skin and abdominal muscle. The portal vein was temporarily clamped except for one branch in order to selectively infuse the islets to specific lobes. The islets were packed in a butterfly-needle as described above and then infused to the portal vein in a volume of approximately 100 µl. The clamp was then removed and the portal vein was compressed with a small piece of Spongostan ® (Johnson & Johnson AB, Sollentuna, Sweden) for 1 minute in order to prevent bleeding. The abdominal muscle and skin were closed with sutures.
Metabolic Evaluation Post-transplantation (Papers I-IV)

After islet transplantation, blood glucose concentrations were monitored daily for the first week and then every fifth day. Blood was collected from the tip of the tail and blood glucose was measured with glucose reagent strips (Freestyle Lite).

One month post-transplantation an intravenous glucose tolerance test (IVGTT) was performed after assessment of the baseline blood glucose concentration. Glucose (300 mg/ml, 2 g/kg (paper I, III and IV) or 2.5 g/kg (paper II), Fresenius Kabi, Uppsala, Sweden) was injected in the tail vein of awake animals and blood glucose concentrations was measured after 10, 20, 30, 60 and 120 minutes.

In paper IV, an intravenous insulin tolerance test (ITT) was performed in addition to the IVGTT on a separate day. The baseline blood glucose concentration was assessed and insulin (NovoRapid ©, 2U/kg, diluted in saline, Novo Nordisk, Bagsvaerd, Denmark) was injected in the tail vein of awake animals. Blood glucose concentrations were measured after 10, 20, 30, 60, 90 and 120 minutes.

Since the graft bearing organs (i.e. abdominal muscle, greater omentum and the liver) were not surgically removed at the end of the study, a piece of the endogenous pancreas was retrieved, weighted, and sonicated in 1 ml acid ethanol (0.18 mol/L HCl in 95% (vol/vol) ethanol). The insulin content of the extract was measured with an insulin enzyme-linked immunosorbent assay (ELISA) (Mercodia, Uppsala, Sweden) in order to exclude endogenous beta-cell regeneration. An insulin content >15% of that of the pancreas of separate control animals was used as an exclusion criterion.

Immunohistochemistry (Papers I-V)

Tissues were either fixed in 10% (vol/vol) buffered formalin before paraffin embedding, or embedded in TissueTek OCT (Sakura Finetek, Torrance, CA, USA) and frozen. Sections were prepared at a thickness of 4 µm for paraffin embedded tissue and 8 µm for frozen tissue and mounted on poly-L-Lysine slides (Thermo Scientific, Braunschweig, Germany).

Immunohistochemistry on frozen sections:

Beta-cell hypoxia was determined by double-staining for insulin (Insulin A, Santa Cruz, CA, USA; dilution 1:100) and pimonidazole (Omni kit, Hypox-yprobe, Burlington, MA, USA; dilution 1:100). Alexa fluor 488 (donkey anti-goat, highly cross-absorbed) and 594 (donkey anti-rabbit) (Invitrogen, Carlsbad, CA, USA; both diluted 1:1000) were used as secondary antibodies.
Beta-cell death by apoptosis was determined by double-staining for insulin (Insulin A, Santa Cruz) and cleaved caspase-3 (Cell Signaling Technology, Beverly, MA, USA; dilution 1:600) using the same secondary antibodies as for insulin and pimonidazole.

Macrophages were stained with F4/80 (eBioscience, San Diego, CA, USA; rat anti-mouse, dilution 1:200) as the primary antibody and Alexa Fluor 555 (donkey anti-rat, Invitrogen; dilution 1:1000) as the secondary antibody.

Complement C3 (Abcam, Cambridge, UK, rabbit polyclonal, dilution 1:20) was double-stained with insulin (guinea pig polyclonal; dilution 1:400; Fitzgerald, Acton, MA, USA) and secondary antibodies used were Alexa Fluor 488 (goat anti-guinea pig, highly cross-absorbed) and Alexa Fluor 594 (goat anti-rabbit; both diluted 1:300; Invitrogen). Nuclei were stained with Hoechst 33342.

In paper I, islet vascular density was determined by double-staining for insulin (Dako, Glostrup, Denmark, polyclonal guinea pig; dilution 1:300) and CD31 (BD Biosciences, Franklin Lakes, NJ, USA; dilution 1:50). Alexa Fluor 555 (goat anti-rat) and Alexa fluor 488 (goat anti-guinea pig) (Invitrogen; both diluted 1:1000) were used as secondary antibodies.

The contribution of donor endothelium in revascularization of islets transplanted to the omentum was determined in paper III in animals transplanted with islets isolated from Flk1-GFP expressing mice. Sections of islet grafts were stained for insulin (Dako, dilution 1:300), CD31 (rat monoclonal; dilution 1:50; BD Pharmingen; San José, CA, USA) and GFP (rabbit polyclonal; dilution 1:200; Invitrogen). Alexa fluor 633 (goat anti-guinea pig), Alexa Fluor 555 (goat anti-rat) and Alexa Fluor 488 (goat anti-rabbit) (all diluted at 1:1000; Invitrogen) were used as secondary antibodies. The staining protocol was confirmed on pancreatic sections from Flk1-GFP mice.

All primary antibodies were incubated with tissue sections in a humidified dark chamber at 4 °C over-night, whereas the secondary antibodies were incubated in room temperature for 1 hour. ProLong ® Gold Antifade reagent with DAPI (Life Technologies, Rockville, MD, USA) was used to mount slides and for nuclei staining on cryosections.

**Immunohistochemistry on paraffin embedded sections:**

In paper III, islet vascular density was determined by double-staining for insulin (dilution 1:400, Fitzgerald) and biotinylated Bandeiraea Simplifolia agglutinin-1 (Bs-1; dilution 1:100; Sigma-Aldrich). The insulin antibody was detected by MACH 3 Rabbit HRP-Polymer Detection (Biocare Medical, Concord, CA, USA) and visualized by 3,3′-diaminobenzidine, whereas Bs-1 was detected with Trekavidin and visualized by Vulcan fast red (Biocare Medical) as previously described (166). All sections were counterstained with hematoxylin before mounting.
Islet nerve density in paper III was determined by double-staining for insulin (dilution 1:400; Fitzgerald) and neuron-specific class III beta-tubulin ((TUJ-1), polyclonal chicken, dilution 1:400; RayBiotech, Norcross, GA, USA). Secondary antibodies used were Alexa Fluor 488 (goat anti-guinea pig) and Alexa Fluor 555 (goat anti-chicken; both diluted 1:250; Invitrogen). Nuclei were stained with Hoechst 33342.

To determine the contribution of donor endothelium in revascularization of human islets transplanted to the omentum in paper III, sections of human islet grafts were double-stained with insulin (dilution 1:400, Fitzgerald) and a CD31/PECAM-1 antibody specific for human CD31 (polyclonal sheep, dilution 1:100, R&D Systems, Minneapolis, MN, USA). Alexa Fluor 555 (goat anti-guinea pig) and Alexa Fluor 488 (donkey anti-sheep) (both diluted 1:250, Invitrogen) were used as secondary antibodies and nuclei were stained with Hoechst 33342. The staining protocol was confirmed on human pancreatic sections.

In paper V consecutive sections of paraffin embedded intramuscular islet grafts were prepared and every other section was stained for insulin (dilution 1:400; Fitzgerald) as described above.

In paper IV and V, sections were double-stained for insulin (dilution 1:400; Fitzgerald) and glucagon (mouse monoclonal, dilution 1:800, Abcam, Cambridge, UK). Secondary antibodies used were Alexa Fluor 488 (goat anti-guinea pig) and Alexa Fluor 555 (donkey anti-mouse; both diluted 1:250; Invitrogen). Nuclei were stained with Hoechst 33342.

Microscopy (Papers I-V)

For every analysis, at least 5 sections from each animal were evaluated. Fluorescent immunohistochemistry images were acquired with a Nikon Eclipse C1/TE-2000U (Nikon Instruments, Amsterdam, Netherlands) or a Zeiss LSM780 (Zeiss, Jena, Germany) confocal microscope. The images were evaluated with Imaris © (Bitplane AG, Zurich, Switzerland) or Image J (NIH, Bethesda, MD, USA). Light microscopy images were acquired with a Leica LMD6000 laser microdissection microscope (Leica Microsystems).

Evaluation of Beta-Cell Hypoxia (Papers I & III)

Beta-cell hypoxia post-transplantation was evaluated by an intravenous injection in the tail vein of the biochemical marker pimonidazole (60 mg/kg body weight, Omni kit, Hypoxyprobe). Two hours post-injection, the animals were anesthetized with sodium pentobarbital (Apoteket) and the graft bearing organ and pancreas were harvested. The tissue was frozen in tissue blocks and cryosections of 8 µm thickness were prepared and double stained
for insulin and pimonidazole, for antibody details see Immunohistochemistry. Fluorescent images were acquired with a confocal microscope. The pimonidazole and insulin positive areas were determined by the computer software Imaris © (Bitplane AG) based on a fixed fluorescent intensity threshold value. The results were expressed as the percentage of pimonidazole positive beta-cell area. An average was calculated for all the images evaluated in the same animal and was considered as one experiment.

Pimonidazole, or 2-nitroimidazole, belongs to the chemical nitro-aromatic compounds and seldom occur naturally. However, most organisms and mammalian cells can metabolize 2-nitromidazole by nitroreductase enzymes, such as aldehyde oxidase and DT-diaphorase in the cytoplasm, NADPH-cyotchrome reductase and cytochrome P-450 in the microsomes and dihydrolipoamide dehydrogenase and succinate dehydrogenase in the mitochondria (167). These enzymes reduce the nitro-group of 2-nitromidazole, but this step is inhibited and rapidly reversed by oxygen. Therefore, in cells with a normal oxygen tension pimonidazole is not further metabolized but instead oxidized and does not accumulate within the cell. However, if the oxygen tension is below 10 mmHg the first reductive step is followed by a number of one electron reductive steps, which lead to the formation of reactive adducts that bind to macromolecules within the cell (168, 169). The side-chains of these 2-nitromidazole adducts can then be detected by antibodies (170) and therefore assessed by immunohistochemistry, ELISA or Western Blot. Since the reduction of pimonidazole depends on an active enzymatic process, it does not accumulate in dead or necrotic cells (171). In dispersed pancreatic islet cells pimonidazole has been shown to accumulate in cells with an oxygen tension below 10 mmHg (172).

Evaluation of Beta-Cell Apoptosis (Paper I)

In order to evaluate the number of cells undergoing apoptosis, all insulin positive cells, and the number of insulin positive cells positive for Caspase-3, were manually counted using Image J (NIH). Data were expressed as percentage of cells undergoing apoptosis. An average was calculated for all the images evaluated in the same animal and was considered as one experiment.

Vascular and Neural Density (Papers I and III)

In paper I and III, vascular density was determined by measuring the area positive for CD31 in contact with beta-cells. The area was divided by the insulin positive area. In paper III the neural density was determined in both mouse and human pancreatic islets. Nerves were stained with the general neural marker TUJ-1 (see Immunohistochemistry). The density was deter-
mained by dividing the area of TUJ-1 positive cells in contact with beta-cells by the insulin positive area. The area of vessels, nerves and beta-cells was determined by the computer software Imaris © (Bitplane AG) based on a fixed fluorescent intensity threshold value.

In paper III, vascular density was also determined by manually outlining insulin and Bs-1 positive areas in mouse and human pancreatic islets in a Leica LMD600 microscope using the Leica Microdissection System software version 7.5.1.5250 (Leica Microsystems, Wetzlar, Germany). An average was calculated for all the images evaluated in the same animal and was considered as one experiment.

**Evaluation of Donor Endothelium Contribution in Islet Revascularization (Paper III)**

The contribution of donor endothelium in revascularization of islets transplanted to the omentum was determined in both mouse and human islets. Islets isolated from Flk1-GFP mice (which express GFP in endothelial cells) were transplanted to the greater omentum of C57BL/6 recipients. The grafts were surgically retrieved and fixed in sucrose to preserve the vascular integrity, and then frozen. Cryosections were triple stained with primary antibodies binding to insulin, CD31 and GFP (for antibody details see **Immunohistochemistry**). The area of vessels and beta-cells was determined by the computer software Imaris © (Bitplane AG) based on a fixed fluorescent intensity threshold value. By such means, the total vascular density could be assessed by dividing the area of CD31 positive cells with the insulin positive area. Next, the number of CD31 positive cells that were also positive for GFP (i.e. of donor origin) was determined.

Grafts of human islets transplanted to the greater omentum of C57BL/6 nu/nu mice were retrieved and double stained for insulin and a CD31/PECAM antibody specific for human endothelial cells. As described above, the insulin positive area was determined as well as the area positive for human-specific endothelial cells.

**In vivo Islet Blood Perfusion and Oxygen Tension (Paper III)**

The animals were anesthetized and placed on a heated table to maintain normal body temperature. A polyethylene catheter was placed in the carotid artery for continuous monitoring of blood pressure (ADInstruments, Dunedin, New Zeeland). A midline incision in the skin and muscle was made to expose the pancreas and stomach including the graft-bearing greater omen-
tum. The tissues were embedded in cotton pads soaked in saline in between measurements in order to keep them from drying.

The islet graft in the omentum was then visualized with a fluorescence stereo microscope (transplanted islets express GFP) and fixated using humidified surgical drape and ligatures. A catheter was placed in the right femoral artery. The vasculature and blood flow were visualized by injections of Alexa Fluor 555 or -647 nm conjugated CD31 antibodies (Invitrogen) and rhodamine-dextran 70 kDa (Sigma-Aldrich). Imaging was performed using a Zeiss Plan-Apo 40×/1.0 W objective on a Zeiss 5 Live line-scanning confocal microscope.

**In vivo** blood flow in mouse and human islet grafts in the omentum was measured with laser-Doppler flowmetry (Transonic BLF21 Series, probe diameter 1.2 mm; Ithaca, NY, USA). The measurements using laser-doppler are made possible by the shift in wavelength that the photons undergo when they hit the moving red blood cells. This produce a voltage proportional to the blood flow which can be recorded by photodetectors inside the probe (173). Blood flow values were recorded as arbitrary tissue perfusion units (TPU), since the equipment is not easily calibrated in physical units of blood flow. Due to the size of the probe, measurements of blood flow in endogenous islets could not be performed. Instead, the gastric wall was used as a reference since it is the closest underlying anatomical reference to the islets engrafted in the omentum.

The **in vivo** oxygen tension in endogenous mouse islets, mouse and human islet grafts in the omentum and the gastric wall was measured using custom-made Clark microelectrodes (tip diameter 2-5 µm; Unisense, Aarhus, Denmark). The tip of the electrode was placed in the tissue to be measured by the use of micromanipulators under visual inspection in a microscope. Multiple measurements were carried out for every tissue (≥5 per location) in each animal. A mean of all measurements was calculated and considered to be one experiment. All measurements were performed according to previously established protocols (174).

Microelectrode measurements of oxygen tension are made possible by the unique and characteristic current-voltage curve that occurs when solutions containing electro-oxidizable or electro-reducible substances are electrolyzed in a cell (175). For the characterization of oxygen there is a sigmoidal voltage-current curve with a plateau between 0.5-0.9 V in which the current depends on the pO₂ in the surrounding solution of the cathode surface. Oxygen is immediately reduced at the cathode surface and the current will therefore be limited by the diffusion rate of oxygen to the cathode. The diffusion of oxygen is limited by factors in the electrolyte solution within the electrode shaft, the electron membrane and by the medium outside the membrane. Since the electrolyte solution within the microelectrode and the electron membrane are constant the current is directly proportional to the pO₂ in the tissue surrounding the microelectrode (27, 176). The modified Clark microel-
lectrodes used in these experiments are composed of three electrodes in a glass shaft filled with an electrolyte solution. The sensing cathode is a platinum wire, the guard electrode a silver wire and the reference anode an Ag/AgCl wire. The electrolyte solution consists of 0.5 mol/l KCl buffered with 50 mmol/l K$_2$CO$_3$ and 75 mmol/l KHCO$_3$ (pH 10.2). Functionally, the pO$_2$ electrode consists of two separate circuits, i) the platinum cathode and silver anode which form the true oxygen sensing system and ii) the silver cathode and the silver anode which form the guard system. Both circuits are supplied with -0.8V from separate voltage sources and the current of the oxygen sensitive circuit, i.e. the oxygen tension, is recorded (176). The microelectrodes were calibrated in Na$_2$S$_2$O$_5$ or air at 37°C before and after the experiments.

Gene Expression of Native and Transplanted Islets Retrieved by Laser Capture Microdissection (Paper IV)

Preparation of tissue for laser microdissection
Snap frozen (liquid nitrogen) tissue of graft bearing organs and pancreas were sectioned at a thickness of 10 µm. The cryostat and accessories were cleaned with ethanol followed by 30 minutes of UV-light prior to usage. Every fifth section was stained with hematoxylin to find the islets and, when confirmed, the consecutive sections were mounted on frame slides (POL-Membrane 0.9 µm, Leica Microsystems). The sections were then fixed in ice cold acetone for two minutes, air-dried and stored in RNase free 50 ml tubes (Ambion, LifeTechnologies, Stockholm, Sweden) at -80°C. The frame slides were thawed and stained with RNase free hematoxylin (Applied Biosystems, Foster City, CA, USA) and dehydrated in increasing grade of ethanol. Laser microdissection of the native islets and islet grafts was performed with a Leica LMD6000 microscope using the Leica Microdissection System software version 7.5.1.5250 (Leica Microsystems). The micro-dissected samples were collected in lysis buffer (Qiagen, Hilden, Germany) and vortexed thoroughly before storage at -80°C until proceeding with the RNA isolation step.

RNA Isolation
Total RNA was isolated from the laser micro-dissected samples using RNeasy Plus Micro Kit (Qiagen), following the manufacturer’s instructions. The amount and purity (OD 260/280) of the total RNA were determined with a spectrophotometer (NanoDrop 2000C, Thermo Scientific, Waltham, MA). RNA yields of the LMD samples were in the range of 5-15 ng. All RNA samples had OD 260/280 between 1.9 and 2.1, which is in the range for pure RNA. The extracted total RNA was dissolved in nuclease free water and stored at -80°C until cDNA synthesis.
**cDNA Synthesis**

RNA was transcribed to cDNA by Superscript First-Strand Synthesis Super Mix for qRT-PCR (Invitrogen, Life Technologies, Stockholm, Sweden) according to the manufacturer’s instructions. The cDNA was stored at -20°C until use for qPCR. Two independent reverse transcriptase reactions were carried out for each RNA sample.

**Amplification of cDNA**

cDNA was amplified using RealTime ready cDNA Pre-Amp Master kit (Roche Diagnostics) with a 4x Primer Pool of 180 nmol/l of each of the primer-pairs. Pre-amplification was performed according to the manufacturer’s instructions at 60°C for 14 cycles and then diluted 1:20 prior to qPCR. The RNA samples were pre-amplified twice in order to confirm the stability and reproducibility of the amplification method.

**Primers**

Primer pair selection criteria for; glucokinase (*GCK*), glucose transporter 2 (*GLUT2*), mitochondrial glycerol-3-phosphate dehydrogenase (*GPD2*), lactate dehydrogenase A (*LDHA*), pyruvate carboxylase (*PCX*), pancreatic and duodenal homeobox gene 1 (*PDX1*), insulin1 (*INS1*), insulin2 (*INS2*) and the reference genes; glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), ribosomal protein S7 (*RPS7*), hypoxanthine guanine phosphoribosyl transferase (*HPRT*) were set to generate short amplicons of 78-182 base pairs (bp) with an annealing temperature of 60°C and without predicted dimer formation using Primer BLAST (NCBI). Primers for *INS1*, *INS2*, *HPRT* were purchased from Tebu-Bio (Roskilde, Denmark); Catalog no *INS1*; MQP027447, *INS2*; MQP027448 and *HPRT*; MQP030898) whereas all other primers were purchased from Sigma-Aldrich.

**Quantitative real-time PCR**

qPCR was performed with a Light Cycler 480 (Roche Diagnostics) and Light Cycler FastStart DNA Master PLUS SYBR Green I kit (Roche Diagnostics). All qPCR samples were run in duplicates. The expression stability of reference transcripts *GAPDH*, *RPS7* and *HPRT* was evaluated with Normfinder software (177). The best normalization was obtained by using the geometric mean of the expression of the reference genes *GAPDH* and *HPRT*. Results are presented as threshold cycle values (Ct-values). The Ct-values were used to calculate the amount of PCR product compared to reference genes by subtracting the Ct-value for reference genes (ΔCt). Relative mRNA expression was calculated as $2^{-\Delta Ct}$.

**Agarose Gel-Electrophoresis**

qPCR products were analyzed by electrophoresis in order to confirm amplicon size using a 3% agarose gel (PCR-grade, Bio-Rad, Hercules, USA).
Electrophoresis was conducted at an electrical field of 5 V/cm for 80 minutes and the bands were visualized with GelRed (Biotium, Hayward, USA) and detected by the Chemi Doc MP Imaging System (Bio-Rad).

Quantification of Total Insulin and Glucagon Area in Intramuscular Islet Grafts (Paper V)

Consecutive sections of the whole intramuscular islet graft were prepared and every other section was stained for insulin as described in the Immuno-histochemistry section. The insulin area of each stained section was manually assessed by outlining it using the Leica Microdissection System software version 7.5.1.5250 and a Leica LMD600 microscope (Leica Microsystems). The number of sections was plotted on the x-axis and the known insulin areas on the y-axis and the total insulin area was then calculated as an area under the curve (AUC) using GraphPad Prism version 6 (GraphPad Software, La Jolla, CA, USA).

The total glucagon area was estimated based on the calculated total insulin area. From separate sections from the same animals the percentage of glucagon positive area in relation to the insulin positive area was calculated. The respective areas were determined by the computer software Imaris © (Bitplane AG) based on a fixed fluorescent intensity threshold value. For each image the glucagon positive area was divided by the insulin positive area and expressed as a percentage. An average percentage of glucagon area was calculated for each animal and multiplied with the respective calculated total insulin area in order to give the estimated total glucagon area.

Ex vivo organ distribution studies (Paper V)

Non-diabetic mice transplanted with varying numbers of islets (100 – 400 islets) to the abdominal muscle were administered 0.2-0.5 MBq $[^{177}\text{Lu}]$Lu-DO3A-VS-Cys40-Exendin-4 intravenously in the tail vein. The animals were euthanized by CO$_2$ 60 minutes after injection of the tracer. Blood, heart, lungs, liver, pancreas, spleen, kidney, intramuscular islet grafts, urinary bladder, subcutaneous fat, muscle, bone, small- and large intestine (without content) were surgically retrieved, weighed and measured for radioactivity in a well-counter (Uppsala Imanet, GE Healthcare, Uppsala, Sweden). The radioactive uptake in each tissue was expressed as Standardized uptake value (SUV), according to equation 1.

$$SUV = \frac{Radioactivity_{\text{organ}}(\text{MBq}) \times weight_{\text{mouse}}(g)}{Radioactivity_{\text{injected}}(\text{MBq}) \times weight_{\text{organ}}(g)}$$ (1)
The total radiotracer uptake in each graft was also assessed as the percentage of injected dose (%ID) by calculating the total uptake in muscle biopsies containing islet grafts \( (\text{Bq}/\text{cc}_{\text{graft}} \times \text{Graft biopsy weight}) \) and subtracting the calculated background concentration of the tracer in a pure muscle biopsy of identical weight \( (\text{Bq}/\text{cc}_{\text{muscle}} \times \text{graft biopsy weight}) \).

**Autoradiography (Paper V)**

Autoradiography experiments were performed on pancreatic tissue and islets transplanted to muscle which were either frozen and sectioned on the same day as the injection of \( [^{177}\text{Lu}]\text{Lu-DO3A-VS-Cys40-Exendin-4} \) or paraffin embedded sections which were prepared and analyzed within six days \( (t_{1/2} \text{ of Lutetium-177 (}^{177}\text{Lu}) \) after injection. The presence of islets was confirmed on adjacent sections by hematoxylin staining in order to select which sections to use for autoradiography. A reference of a \( ^{177}\text{Lu} \) with known radioactivity was included in order to enable quantification of islet uptake in Bq/pixel. By using the known specific radioactivity of the tracer \( (\text{fmol}/\text{Bq}) \) the receptor binding in each islet \( (\text{fmol}) \) could be calculated. After exposure, each phosphor screen was scanned by using a Cyclone phosphorimage (PerkinElmer, Waltham, MA, USA) with a resolution of 600 dpi. The acquired images were analyzed using ImageJ (NIH).

**Clinical PET/CT Examinations**

The clinical study presented in paper VI was approved by the regional ethical board of Uppsala (EPN 2011/439). All study participants signed a written informed consent prior to any investigations within the study. The study was conducted in accordance with the Declaration of Helsinki.

Patients with T1D \( (n=10) \) and healthy controls \( (n=9) \) were fasting for >4 hours prior to the PET/CT-examination. Insulin doses were adjusted for the patients with T1D in order to maintain blood glucose concentrations between 3-12 mmol/l during the PET-examination. Prior to the administration of \( [^{11}\text{C}]5\text{-HTP} \), a single dose of 15-oxygen-labeled water \( ([^{15}\text{O}]\text{WAT}) \) was administered to determine the pancreatic blood perfusion. After that 2-5 MBq/kg of \( [^{11}\text{C}]5\text{-HTP} \) was administered and the tracer uptake was studied for 60 minutes with a dynamic PET-protocol. The healthy controls had undergone a mixed-meal tolerance test (MMTT) prior to the PET-examination in order to exclude glycemic intolerance. All patients with T1D had undetectable levels of C-peptide \( (<0.003 \text{ nmol/l}) \). Two out of the ten patients with T1D had previously received a pancreas transplant but lost graft function and displayed HbA1c-levels and insulin needs comparable to those prior to transplantation. In the study their native pancreas was assessed.
A retrospective analysis of patients (n=10) who had undergone repeated $[^{11}C]5$-HTP PET/CT-examinations for the investigation of neuroendocrine tumors (NETs) was also assessed. Two of the patients were diagnosed with T2D, whereas the remaining patients had no reports on impaired glycemic control. Similar to the cross-sectional study, the patients received 2-5 MBq/kg $[^{11}C]5$-HTP, but the PET-examination protocol only lasted for 25 minutes. In addition, the patients received 100-200 mg carbidopa orally prior to the examination. Therefore, the data from the retrospective study were not fully comparable to those of the patients investigated in the cross-sectional study.

Statistics

Statistical analysis was performed using GraphPad Prism version 6 (GraphPad Software). All values are given as mean ± standard error of mean (SEM). An unpaired two-tailed $t$ test was used to compare differences between two groups. Differences between several groups were determined by one-way ANOVA. In paper I, Fisher’s LSD post-hoc test, or when comparison of treatment groups to control, Dunnet’s post-hoc test was applied. In paper II, III and IV Tukey’s post-hoc test was used. In Paper IV a non-parametric one-way ANOVA with Dunn’s post-hoc test was applied to compare the relative gene expression of transplanted islets with that of native islets. For all comparisons, p values <0.05 were considered statistically significant.
Results and Discussion

Beta-Cell Hypoxia in Transplanted Islets

A substantial degree of beta-cell hypoxia was observed on the first day following intramuscular islet transplantation in paper I. Approximately 80% (77±3%) of the insulin positive part of the islet graft was positive for pimonidazole, i.e. hypoxic. The degree of hypoxia decreased effectively over time, after four days 36±5% and after seven days only 17±5% of the beta-cells suffered from hypoxia. In fact, after 14 days post-transplantation the number of beta-cells suffering from hypoxia was close to zero (0.30±0.08%). The decrease in beta-cell hypoxia coincided with the restoration of the islet vascular network. It has previously been shown that capillaries can be found within the islets already after five days and that the vascular network is fully restored and comparable to that of native islets after 14 days (80). However, it should also be noted that pimonidazole does not accumulate in dead or dying cells (171).

By co-transplanting islets with PolyHb in increasing doses, the acute beta-cell hypoxia could effectively be reduced. Beta-cell hypoxia one day post-transplantation was effectively reduced to 25±4% with use of the low dose, 16±2% with the medium dose and to 4±1% with the high dose of PolyHb.

Beta-cell hypoxia in islets transplanted to the greater omentum was also found to be substantial on the first day post-transplantation (47±7%) in paper III, but not as pronounced as in islets transplanted to muscle. As observed at the intramuscular site, the degree of hypoxia rapidly declined and after four days only 16±4% of the beta-cells was hypoxic. Already after seven days the percentage of hypoxic beta-cells was down to 4±1%.

Previous studies of islets transplanted to the liver have demonstrated that the degree of hypoxia remains high in beta-cells even one month post-transplantation due to slow graft revascularization (70, 178). All islet grafts, regardless of transplantation site, are relying on oxygen diffusion until a new vascular network has been restored, since end-to-end vessel anastomoses cannot be surgically performed as in solid organ transplantation. Therefore, the low oxygen tension in the liver as compared with muscle and the omentum probably contributes to this prolonged ischemia in the liver in combination with the slow revascularization.

Dionne et al showed in an in vitro study that even a mild reduction of the oxygen tension (27 mmHg) impairs the second-phase of insulin secretion in
freshly isolated islets by up to 50%. However, when the islets have been cultured a second-phase of insulin secretion $\geq 50\%$ of that observed during normoxia is maintained down to an oxygen tension of 10 mmHg (30). Therefore, it is likely that the hypoxia and poor revascularization in islets transplanted to the liver not only contributes to beta-cell death but also to beta-cell dysfunction.

**Beta-Cell Apoptosis in Intramuscular Islet Grafts**

Beta-cell apoptosis was found to be highest on the first day post-transplantation (5.7±0.4%). Apoptosis rates were reduced by almost 80% after four days (1.3±0.2%) and then remained at low levels. A loss of islet cytoarchitecture, preferentially in the center of the islets, indicated that necrosis also contributed to beta-cell death. When islets were co-transplanted with low-dose PolyHb, the apoptosis rates were halved (2.9±0.5%). Despite the effective reduction of beta-cell hypoxia with high-dose PolyHb, there was no effect on beta-cell apoptosis and a more severe disruption of the islet architecture was observed. In the animals transplanted with medium- and high-dose of PolyHb, more macrophages were observed when compared with control and low-dose PolyHb islet grafts.

**Islet Revascularization**

The vascular density in intramuscular islet grafts implanted into alloxan-diabetic mice was similar in control islet grafts and in islets co-transplanted with low-dose PolyHb (7.9±0.7% vs. 9.0±0.9%) in paper I. In mouse islets transplanted to the omentum (paper III), there were vessels penetrating the islet mantle already after seven days. After 14 days the vascular density was half (4.2±0.7%) of that observed in endogenous mouse islets (8.5±0.6%) and after one month the vascular density was fully restored (9.8±0.7%). Likewise, the vascular density in human islets transplanted to the omentum was found to be maintained one month post-transplantation compared with endogenous human islets (5.3±0.4% vs. 4.4±0.1%). No, or very limited, contribution of donor endothelium in the vascular network was observed one month post-transplantation for mouse and human islets transplanted to the omentum.

Hypoxia is a key regulator of the angiogenic process and revascularization. Islet transplantation beneath the renal capsule increases the islet expression of angiogenic factors such as hypoxia inducible factor-1 (HIF-1$\alpha$), vascular endothelial growth factor-A (VEGF-A) and hepatocyte growth factor (179-181). VEGF has been shown to be the main angiogenic factor for the vascularization and revascularization of pancreatic islets (18, 182-185). Suc-
cessful reversal of hypoxia in the early post-transplantation phase could therefore potentially impair the highly effective revascularization process of islets at the intramuscular site. However, there were no differences in the degree of revascularization between islets co-transplanted with the low dose of PolyHb and control islets.

Omentum is unique in that it demonstrates the highest VEGF secretion rate, as well as the highest concentration of VEGF protein of various tissues and organs examined (186). In fact, the VEGF protein content in the omentum is more than 200 times higher than in the liver, spleen or other abdominal organs, and may increase even further in response to hypoxic signaling (186). In addition, beta-cells also express VEGF which in the native pancreas stimulates the maintenance of a high vascular density and is of importance for revascularization in the transplantation setting (18, 182). It is therefore not surprising that islets transplanted to the omentum become well vascularized and that the vascular density is comparable to that of endogenous islets.

### The Role of Complement Activation in Intramuscular Islet Transplantation

By immunohistochemistry a large number of cells positive for complement C3 could be detected within and surrounding islets transplanted to muscle one day post-transplantation. However, no or very limited, activation was observed by immunohistochemistry one month post-transplantation. Despite the activation of complement in the acute phase, there was no difference in the time to normoglycemia or islet graft function one month post-transplantation when comparing animals deficient of complement C3 with control animals. Not even when both the isolated islets and recipient animals were of C3 -/- origin could a difference be observed. This indicates, despite the initial activation, that the activation of complement in islets transplanted to muscle does not hamper the islet graft function.

The complement system is a part of the innate immune system and an important defense mechanism against pathogens. There are three pathways for activating the complement system; i) the classical-, ii) the lectin- and iii) the alternative pathway. The key regulator for all pathways is the complement factor C3. After the cleavage and activation of C3 to C3a the complement cascade proceeds and leads to the formation of a membrane attack complex (MAC) which causes lysis of the cell-membrane and cell death. Components of the complements system are mainly produced in the liver and then secreted to the circulation. However, complement factors are also produced locally in many other tissues, such as muscle and endothelium (187). It has also been demonstrated that complement factors are produced in the pancreas and
secreted into the exocrine fluid (188). Deposits of complement can be found in excess amount in the exocrine pancreas of patients with T1D (189). In addition, beta-cells express complement C3 at the mRNA level (190), but the potential role of complement activation in the beta-cells has not been studied in detail.

In a study of isogenic experimental kidney transplantation in C3 -/- mice, it was shown that complement activation has an important role in kidney damage post-transplantation and especially in ischemia-reperfusion damage (191). Regardless of the recipient origin acute kidney failure and severe fibrosis developed if the transplanted kidney was C3-positive and suffered from ischemia prior to transplantation. However, when C3-negative kidneys were transplanted, the functional and structural impairments were mild, even when transplanted into wild-type animals (191). This suggests that the activation of complement is not only restricted to the recipient, but also, and maybe even more importantly, occurs in cells from the transplanted tissue.

Islets transplanted to muscle do suffer from hypoxia/ischemia (paper I). However, no beneficial effect was observed when transplanting C3-negative islets. One possible explanation could be that the islets, which are re-vascularized instead of surgically anastomosed to the systemic circulation, do not suffer from reperfusion injuries which in the case of kidney transplantation seemed to be the main trigger for complement activation (191).

Blood Flow and Oxygenation in Native and Islets Transplanted to the Omentum

At 14 days post-transplantation a functional glomerular-like vascular network with perfused blood vessels could be visualized in mouse islets transplanted to omentum in vivo. The blood flow measured in both mouse and human islets by laser-Doppler flowmetry one month post-transplantation was found to be 2.5 times higher than the blood flow in the adjacent gastric wall and similar to that previously recorded in the well vascularized renal cortex (192). The oxygen tension recorded in the transplanted mouse and human islets were similar to that of endogenous mouse islets. Previous studies have shown that the oxygen tension in intramuscular islet grafts is superior when compared to islets transplanted beneath the renal capsule site and close to that of endogenous islets (193).

A sufficient blood flow of native and transplanted islets is obviously of great importance for the delivery of oxygen and nutrients, and also in order to provide an adequate route for the delivery of secreted hormones (194).
Innervation of Islets Transplanted to the Omentum

The nerve density in endogenous islets and in islets transplanted to the omentum was assessed by the general neural marker TUJ-1, which stains both parasympathetic and sympathetic nerves. The nerve density in endogenous mouse islets was found to be 1-2‰ and was maintained in mouse islets transplanted to the omentum one month post-transplantation. In endogenous human islets the nerve density was ~4‰, and was also maintained in transplanted human islets one month post-transplantation.

In mouse islets transplanted beneath the renal capsule, neurons, both sympathetic and parasympathetic, were found within the islets after six weeks, but re-innervation was not fully established until 14 weeks post-transplantation (195). The re-innervation pattern of transplanted islets has been found to be dependent on the implantation site. In a comparison of islets transplanted to the liver, spleen and beneath the renal capsule in mice, there was a dominance of sympathetic neurons in islet grafts in the liver and spleen, whereas both sympathetic and parasympathetic neurons were found in islet grafts beneath the renal capsule. In fact, the re-innervation pattern of the islet grafts seemed to reflect the innervation pattern of the implantation organ (93).

In studies comparing the innervation of human and mouse pancreatic islets a discrepancy become apparent. It seems that in human pancreatic islets parasympathetic neurons are sparse and the sympathetic neurons mainly innervate blood vessels within the islets. This suggests that the neural factors in human islets do not act directly on endocrine cells, but rather on the vasculature by controlling blood flow and/or in a paracrine fashion (196, 197). In animal models it has for long been known that sympathetic and parasympathetic manipulation affects the islet blood flow (194). However, there are species differences even among rodents, which make it very difficult to extrapolate the results to humans. Therefore, the exact pattern of innervation and mechanism for the neural control of hormonal secretion from human pancreatic islets is not fully understood.

In general, the parasympathetic neurons stimulate insulin secretion in response to hyperglycemia and glucagon secretion in response to hypoglycemia. Sympathetic nerves stimulate glucagon secretion in more pronounced hypoglycemia and inhibit insulin secretion (198). In addition to the regulation of hormonal secretion, neural factors, at least in rodents, impact the proliferation of beta-cells as well (199, 200).

Gene Expression of Transplanted Islets

The expression of GLUT2, PCX, PDX1, INS1 and INS2 was upregulated in mouse islets transplanted to both muscle and omentum when compared to
native islets. In contrast, the gene expression of LDHA was down-regulated at both implantation sites. In addition GPD2 was upregulated in islets transplanted to muscle. The expression of GCK was unaltered in both islets transplanted to muscle and omentum.

GLUT2 is a high-capacity, low-affinity and bi-directional glucose transporter which is expressed preferentially in beta-cells (201). In striated muscle and adipose tissue, the insulin regulated glucose transporter GLUT4 is instead predominant. All in all, there are 14 known glucose transporters in humans (201). Due to the different function and affinity of the glucose transporters it is of major importance in transplanted islets that they maintain the expression of GLUT2 in order to function properly. In normal beta-cell physiology the transport of glucose by GLUT2 is however not a rate limiting step due to the high-capacity of the transporter. Instead, the phosphorylation of glucose to glucose-6-phosphate by GCK limits the rate of glucose metabolism in the beta-cells (202). GCK is expressed in beta-cells and hepatocytes, the expression is regulated by glucose in beta-cells and insulin in the hepatocytes (203). The fact that the expression of GCK was unaltered in the transplanted islets is at least indicative of a sufficient phosphorylation of glucose in the transplanted beta-cells. Given also that the expression of INS1 and INS2 was increased further supports a normal beta-cell function in the islet grafts. However, the increased expression of INS1 and INS2 could also reflect a marginal islet mass and thereby increasing demand for insulin secretion from each individual beta-cell. The volume of the graft (200 islets) in fact only represents a portion of that normally found in the native mouse pancreas (150, 204).

Glucose-6-phosphate is further metabolized to pyruvate and due to the low concentration of LDHA, only small amounts of pyruvate are metabolized to lactate (202). Instead, most of the pyruvate is metabolized in the mitochondria and transformed to acetyl coenzyme A by pyruvate dehydrogenase or into oxaloacetate by PCX. In the transplanted islet grafts the expression of PCX was increased whereas LDHA was decreased. Probably these changes are linked to each other, however it is not possible to distinguish based on these data whether the increased PCX caused or is a consequence of the decrease in LDHA and vice versa. Particularly for intramuscular islet grafts, the low expression of LDHA may be of importance, since an increased expression would most probably have forced the transplanted beta-cells to secrete insulin not only in response to increased glucose concentrations but also to lactate generated by myocytes during exercise (205).

In the metabolism of glucose-6-phosphate to pyruvate, oxidized nicotinamide adenine dinucleotide (NAD\(^+\)) is required and in order to re-oxidize the reduced form (NADH) the beta-cell rely on the mitochondrial electron transport chain in which GPD2 plays a key role (206). The mRNA expression of this mitochondrial enzyme was increased in the intramuscular islet
grafts and maintained at endogenous levels in islets transplanted to the omentum.

PDX1 is of major importance both for the development of the pancreas during embryogenesis and for maintenance of normal beta-cell function in adults (207). At least in vitro PDX1 can also stimulate the expression of GLUT2 (208) and insulin (209). The increase in PDX1 in the transplanted islets could therefore, at least in part, explain the increased expression of both GLUT2 and insulin.

Both in islets transplanted into the liver and to the pancreas the expression of PDX1 and GCK have been found to be decreased (79). Furthermore, the expression of INS1 and INS2 was decreased in islets transplanted beneath the renal capsule (210). In addition, in islets transplanted to the pancreas and beneath the renal capsule the gene expression of LDHA was increased (79, 210).

Taken together, the expression profile of genes important for normal beta-cell function is favorable in both islets transplanted to muscle and the omentum when compared with endogenous islets and when compared with published data of islets transplanted to other implantation sites.

Islet Graft Function

Alloxan-diabetic mice were transplanted with intramuscular islet grafts consisting of either 200 or 300 islets alone, or 200 islets co-transplanted with low-dose PolyHb (Paper I). The low-dose PolyHb was selected based on its ability to both reduce beta-cell hypoxia and apoptosis. Animals co-transplanted with PolyHb had lower blood glucose five days post-transplantation when compared with animals transplanted with 200 islets alone. One month post-transplantation the animals were challenged with an IVGTT and the animals co-transplanted with PolyHb displayed a lower glucose 60-minutes post-injection and an improved overall response (expressed as an AUC) when compared with animals transplanted with 200 islets alone. In fact, the overall response to the IVGTT in the co-transplanted animals was comparable to that of the animals transplanted with 300 islets alone.

There was no difference in the time to normoglycemia in alloxan-diabetic mice transplanted with 300 mouse islets to the omentum as compared with the liver. However, when challenged with an IVGTT one month post-transplantation, the animals with islets transplanted to the omentum normalized their blood glucose values within two hours, whereas animals transplanted with islets to the liver were glucose intolerant. An overall better response to the IVGTT (expressed as AUC) was observed in the animals with islets implanted to the omentum when compared with animals transplanted with islets intraportally.
Quantification of Beta-Cell Mass after Intramuscular Islet Transplantation

There was major uptake and retention of $[^{177}\text{Lu}]$Lu-DO3A-VS-Cys40-Exendin-4 in lungs, pancreas, kidneys and urine, whereas pure muscle had a tracer uptake close to zero (SUV<0.1). Muscle biopsies containing islet grafts had a significantly higher uptake than the muscle background. There was a linear correlation between radioactivity uptake in the graft bearing muscle biopsies and the number of transplanted islets, both for SUV and %ID. However, overlaps of grafts transplanted with different number of islets were observed. This could, at least in part, be explained by differences in beta-cell survival in the islet grafts. Therefore, correlations were calculated separately for the grafts in which the total insulin area was determined. A correlation coefficient with the total insulin area to SUV of 0.96 (p=0.0002) and a correlation coefficient of 0.88 (p=0.0095) to %ID was observed. In contrast, there was no correlation between radioactive signal and the estimated total glucagon area. This indicates that $[^{177}\text{Lu}]$Lu-DO3A-VS-Cys$^{40}$-Exendin-4 has a higher preferential binding to beta-cells. This notion has in fact previously been confirmed in a study of native pancreatic islets (211). This is an important aspect for a PET-tracer intended for beta-cell imaging, since the percentage of beta-cells is variable in human pancreatic islets, ranging from 50-80% of the islet volume (15).

The binding of $[^{177}\text{Lu}]$Lu-DO3A-VS-Cys40-Exendin-4 in islets of Langherans in both native and transplanted islets was confirmed by autoradiography. Transplanted islets could easily be delineated from the muscle background in the graft containing biopsies. The receptor density in native islets was approximately 3.5 times higher than the exocrine background (p<0.0001). However, the islet-to-background contrast was even higher in muscle with approximately a 40 times higher binding ratio (p<0.0001). Importantly, the binding in native and transplanted islets was similar, both when expressed as binding density (fmol/mm$^3$ islet tissue) and as binding per islet equivalent. This is also supported by immunohistochemically staining of the GLP-1 receptor in transplanted islets in previous studies (212).

In this experimental setting, a difference in graft size of 200 islets could be detected, although there were overlaps between the groups. In the clinical setting, the number of transplanted islets is often $\geq 10,000$IEQ / kg body weight. Based on the quantitative binding data, an average islet graft in the clinical setting would have the potential of binding in excess 6 nmol DO3A-VS-Cys$^{40}$-Exendin-4 (given that a similar peptide amount is administered). The clinically significant change in islet graft mass is probably $\geq 10,000$ islets, which may well, based on the extrapolation above, be detected despite the small total volume of the islet graft. In the experimental setting in paper V, $^{177}\text{Lu}$ was used as a convenient replacement nuclide to the positron emitting $^{68}\text{Ga}$ because of its higher image resolution and long $t_{1/2}$. The alteration
in biodistribution and islet specific targeting compared to $^{68}$Ga is negligible (213). Therefore, $^{177}$Lu is a very suitable nuclide in a pre-clinical setting. However, in the clinical setting, $[^{68}$Ga]$Ga$-DO3A-VS-Cys$^{40}$-Exendin-4 would be used due to the physical properties of the nuclide and because of the radiation safety. Also, it has been shown that the relatively low radiation dose enables for 2-4 repeated examinations yearly in the same individual (214), which is of importance for the potential use in continuous follow-up after islet transplantation.

As previously reported, the delivery and uptake of the PET-tracer is dependent on the revascularization of the islet graft (212), and islets transplanted to muscle have an excellent revascularization (80). However, even at the intramuscular site, the vascular density is not restored until 14 days post-transplantation (80). Therefore, Exendin-4 would probably not be useful as a marker of alterations in beta-cell mass in the acute setting following islet transplantation.

Quantification of Native Pancreatic Islets

In the retrospective study of patients with NETs, the pancreas could easily be visualized by $[^{11}$C]5-HTP PET/CT. The pancreatic uptake of $[^{11}$C]5-HTP was higher than in other abdominal organs. There was a large variability among the non-diabetic subjects (close to 4-fold) in pancreatic $[^{11}$C]5-HTP uptake. However, the variability within the same subject between different examinations was low (coefficient of variation 6.7%). Out of the ten patients with NETs two patients were diagnosed with T2D. In these two patients the pancreatic uptake of $[^{11}$C]5-HTP (expressed as % ID / gram tissue) was significantly lower. In one of the patients with T2D, repeated PET/CT-examinations were performed in order to monitor and stage the NET and during this time period (totally 30 months) her diabetes progressed in parallel. Seven months after diagnosis of T2D, medication with glimepiride was initiated, which after 15 months was replaced by insulin treatment. During this time a progressive decline in pancreatic $[^{11}$C]5-HTP uptake was observed. In fact when comparing the first PET/CT-examination with that 30 months after diagnosis of T2D, the pancreatic uptake was reduced by approximately 50%.

In the cross-sectional study, patients with long-standing T1D (n=10) with undetectable plasma C-peptide concentrations (<0.003 nmol/l) were compared with age- and BMI matched healthy controls (n=9). The healthy controls had normal fasting and 2-hour post MMTT concentrations of blood glucose and C-peptide, and normal HbA1c levels. The pancreatic uptake of $[^{11}$C]5-HTP was evaluated during a 60-minute dynamic PET/CT examination. During the very first minutes after tracer injection, a high signal was recorded in kidney cortex, spleen and the pancreas due to their high blood
perfusion. After ten minutes, retention of the tracer was observed in the pancreas of healthy controls, but not in patients with T1D. At the end of the examination (60 minutes), the uptake was low in all abdominal organs except the pancreas of healthy controls and the kidney medulla of all subjects. The high uptake in the kidney medulla is explained by the excretion of \([^{11}\text{C}]\text{HIAA}\), a metabolite of \([^{11}\text{C}]\text{5-HTP}\), into urine.

The pancreatic uptake of \([^{11}\text{C}]\text{5-HTP}\) was quantified (expressed as \%ID) and found to be decreased in patients with T1D throughout the whole examination. The reduction was most prominent after 60 minutes with a mean reduction of 66% in the T1D patients. Furthermore, when the pancreas was divided into its three anatomical regions the reduction was most prominent in corpus (68%, \(p<0.001\)) and cauda (70%, \(p<0.01\)) and to a lesser extent in the caput region (60%, \(p=0.01\)) when compared with the healthy controls.

As previously shown in imaging and autopsy studies, the pancreas volume was decreased in patients with T1D. On an average the pancreatic volume in patients with T1D was only 61% of that in healthy controls (54.9±14.6 cc vs. 89.4±23.5 cc, \(p<0.01\)). In addition to a reduced pancreatic volume in the T1D patients there was also a tendency (\(p=0.068\)) towards a reduced pancreatic blood flow as evaluated by oxygen-15-labeled water.

When correcting the pancreatic uptake of \([^{11}\text{C}]\text{5-HTP}\) to the volume of the pancreas (\%ID / gram tissue), the reduced uptake in patients with T1D persisted. The volume corrected reduction for the whole pancreas was 39% (\(p<0.05\)) and as previously observed most pronounced in corpus (41%, \(p<0.001\)) and cauda (47%, \(p<0.01\)) and to a lesser extent in the caput region (34%, \(p<0.05\)).

5-HTP is a precursor of serotonin and its uptake is facilitated by large amine transporters (LATs), which are present in most cells. However, if not further metabolized, 5-HTP will exit the cell through the same mechanism. The use of \([^{11}\text{C}]\text{5-HTP}\) as a PET-tracer therefore depends on both the uptake and metabolism of the substance. All endocrine cells in the islets of Langerhans can metabolize 5-HTP into serotonin. In a recent study of human pancreatic sections from healthy controls and patients with T1D, serotonin was found to be co-localized with the islets of Langerhans (215). However, beta-cells have a preferential uptake of 5-HTP when compared with the other cell types (216). Despite that, \([^{11}\text{C}]\text{5-HTP}\) is not truly a marker of beta-cell mass but rather a marker of endocrine mass. Based on the uptake of \([^{11}\text{C}]\text{5-HTP}\) in isolated human islets mixed with different ratios of exocrine tissue, the uptake in a pure endocrine sample was estimated to be 14 times higher than in exocrine tissue \textit{in vitro} (215). In rats with induced diabetes the pancreatic uptake of \([^{11}\text{C}]\text{5-HTP}\) has been shown to decline in relation to the remaining beta-cell area (215).

In total there are approximately 1.5 million islets within the human pancreas and the majority of them has a diameter < 50 µm. Only a small number of islets have a diameter close to 300 µm (150). As discussed in \textit{Imaging the
Pancreas and the Islets of Langerhans, this means that the resolution in PET/CT-examinations is not high enough to delineate single islets even if the PET-tracer has a preferential uptake in islets. Therefore, the pancreatic uptake (expressed as %ID) represents the total endocrine signal. The pancreas can be divided into three anatomical regions, and from autopsies it is known that the distribution and islet composition vary somewhat between the different regions. In line with the present findings, the beta-cell fraction has previously been described to be higher in the corpus and caudal part of the pancreas, whereas the caput is richer in PP-cells.

In patients with T2D there are many contributors to the poor metabolic control, including insulin resistance, beta-cell dysfunction but also beta-cell death and hence reduced beta-cell mass (35). In the retrospective study, although limited by the small number of patients, we observed a decreased uptake of $[^{11}C]5$-HTP in patients with T2D. Interestingly, in one patient with T2D, which was examined repeatedly, there was a close to 50% decrease in pancreatic $[^{11}C]5$-HTP uptake in parallel with a worsened metabolic control. In autopsy studies, the reduction in beta-cell mass has been reported to be close to 40% in average (35). However, in patients with a disease duration <5 years, the reduction in beta-cell mass was on an average 24%, whereas the patients with a disease duration >15 years had an average reduction of 54%. In the retrospective case, the observed 50% reduction in pancreatic $[^{11}C]5$-HTP uptake after 30 months could, in light of this autopsy study, seem exaggerated. However, first of all it should be noted that this is only one case and, secondly, in the study by Rahier et al (35) there was a great variation in beta-cell mass among the patients with T2D and in some cases a reduction of 50% even within 5 years of diagnosis was observed (35). In addition, the alpha-cell mass has been described to be unaltered in patients with T2D (217), which is of importance for the use of $[^{11}C]5$-HTP as a surrogate marker of beta-cell mass. However, the feasibility of $[^{11}C]5$-HTP as a marker of beta-cell mass in T2D needs to be further investigated in a larger number of patients and with a prospective study design.

Among the healthy controls, the pancreatic uptake of $[^{11}C]5$-HTP varied and the difference in the subject with the lowest uptake compared with the one with the highest was 3.5-fold. Also, among the non-diabetic subjects in the retrospective study, there was a variation in pancreatic uptake. This is well in line with the theoretical variation in beta-cell mass and with what has been observed in autopsy studies (35).

In the patients with T1D, the pancreatic uptake of $[^{11}C]5$-HTP was on average reduced by 66%, which, in fact, is in line with what might be expected after a complete loss of beta-cells. As discussed in the Introduction, the contribution of beta-cells to the total volume of islets of Langerhans varies a lot in human. In a report on beta-cell fraction in isolated human islets and calculations based on human pancreatic sections, it was reported to vary from 46-75% (218). Based on this report, the average beta-cell fraction in human
islets would be around 60%. This means that a complete loss of beta-cells 
would, in average, reduce the total pancreatic endocrine mass by around 
60%, given that other endocrine cells within the islets of Langerhans remain 
unaltered. In fact, in an autopsy study of patients with long-standing T1D, 
the total volume of endocrine cells was determined and found to be reduced 
by approximately 70% due to the loss of beta-cells (7). In the same study the 
number and volume of other endocrine cells within the islets of Langerhans 
were found to be unaltered in patients with T1D. 

Based on the alterations in endocrine mass in T1D described in autopsy 
studies, it seems as the average reduction of 66% in pancreatic $[^{11}\text{C}]5$-HTP 
in patients with T1D very well could reflect the loss of beta-cells. So despite 
that $[^{11}\text{C}]5$-HTP is not truly a marker of beta-cell mass it may well serve as a 
surrogate marker of changes in beta-cell mass in T1D.
Summary and conclusions

Paper I

- Intramuscularly transplanted beta-cells suffer from prevailing hypoxia in the acute post-transplantation phase.

- Beta-cell hypoxia can be overcome and beta-cell apoptosis reduced by co-transplantation of an oxygen carrier, polymerized hemoglobin.

- Co-transplantation with polymerized hemoglobin does not hamper islet revascularization.

- Co-transplantation with polymerized hemoglobin improves the islet graft function at the intramuscular site.

Paper II

- Complement C3 is activated in islets transplanted to muscle in an isogenic mouse model.

- However, transplantation of C3-deficient islets does not improve the functional outcome, not even if the recipients were C3-deficient.

Paper III

- Beta-cells suffer from prevailing hypoxia also when transplanted to the omentum, but the hypoxia is reversed within the first week post-transplantation.

- Both mouse and human islets transplanted to the omentum of mice become rapidly re-vascularized and the vascular density is comparable to that of the corresponding endogenous islets.
• A functional blood flow and oxygen tension comparable to that of endogenous mouse islets were detected in both mouse and human islets engrafted to the omentum.

• Following transplantation to the omentum mouse islets have a superior graft function when compared with islets transplanted to the liver.

Paper IV

• Islets transplanted to muscle and the omentum have a maintained or increased mRNA expression of genes important for normal beta-cell function.

• Alloxan-diabetic animals were cured by islets transplantation with a fairly low number of islets both when transplanted to muscle and omentum.

• Islet grafts at both implantation sites had similar capacity to handle a glucose- and insulin-challenge.

Paper V

• \([^{177}\text{Lu}]\text{Lu-DO3A-VS-Cys}^{40}\text{-Exendin-4}\) can be used to quantify beta-cell mass following experimental intramuscular islet transplantation.

• Accumulation of \([^{177}\text{Lu}]\text{Lu-DO3A-VS-Cys}^{40}\text{-Exendin-4}\) does not correlate to the transplanted alpha-cell mass.

• The technique may well be transferred to the clinical setting by exchanging Lutetium-177 to a positron emitting Gallium-68 radionuclide.

• The establishment of a clinical non-invasive method for quantification of beta-cell mass would be of great importance in the field of islet transplantation.
Paper VI

- The pancreatic uptake of $[^{11}C]5$-HTP is decreased by two thirds in patients with type 1 diabetes when compared to healthy controls.

- Based on the findings in this clinical report combined with previously published pre-clinical data, it seems as if $[^{11}C]5$-HTP can serve as a surrogate marker for native beta-cell mass *in vivo*.

- The establishment of a clinical method for *in vivo* quantification of beta-cell mass would be of immense importance for increasing our understanding of disease development and for the evaluation of treatments aiming to increase beta-cell mass in type 1 diabetes.


ingen metod för att mäta mängden Langerhanska öar hos levande människor utan all kunskap på det området baseras på obduktionsstudier. Vid typ 2 diabetes orsakas det höga blodsockret av en otillräcklig frisättning av insulin i förhållande till behovet och det som kallas ”insulin-resistens”, d.v.s. en nedsatt känslighet för insulin i fett- och muskel-cellern.

Trots upptäckten av insulin och stora framgångar i tillverkningen av syntetiskt insulin är det nästintill omöjligt att med injektioner av insulin helt efterlikna den normala blodsockerregeeringen. Om en för stor dos insulin ges drabbas man av lågt blodsocker (hypoglykemi) som i värsta fall leder till koma och död. Om insulin inte ges vid typ 1 diabetes får man ett kraftigt förhöjt blodsocker (hyperglykemi) som i sin tur leder till stora vätskeförluster och sjunkande pH-värde i blodet (ketoacidos) som är ett livsotande tillstånd. Dessutom kan kroniska komplikationerna uppstå och det kan drabba i princip alla kroppens organ. Till exempel är diabetes den vanligaste enskilda bakomliggande orsaken till både njursvikt, blindhet och underbensamputation i västvärlden.


År 2000 introducerades det så kallade Edmonton-protokollet som ledde till att 80 % av de patienter som fick en ö-transplantation botades, d.v.s. klarade sig utan insulininjektioner, i upp till ett år. Däremot var det endast 10-15 % av patienterna som fortfarande klarade sig utan insulin efter fem år. Sedan år 2000 har resultaten ytterligare förbättrats och med nuvarande immundämpande behandling klarar sig nästan 60 % av patienterna utan insulin i upp till fem år. Däremot krävs det att man isolerar öar från två till fyra donerade bukspottskörtlar. Det finns således ett stort behov av förbättringar för den enskilda patienten och för att möjliggöra fler transplantationer. En starkt bidragande orsak till de nedslående resultaten är valet av levern som målorgan för transplantation av de Langerhanska öarna. I bukspottskörteln är öarna välförsörjda med kärl och har därigenom god tillgång till både syrgas och näringsämnen. När öarna isoleras från bukspottskörteln förlorar de sin kontakt med kärl och nerver och dessa kan inte återställas kirurgiskt eftersom öarnas genomsnittliga diameter är endast 150 µm. De är därför beroende av att syrgas och näringsämnen når dem från nära angränsande kärl tills dess att nya kärl växer in. I levern är dock tillgången på syrgas låg i jämförelse med öarnas naturliga miljö och det har visat sig i experimentella studier att även kärlåterväxten till öarna är dålig. För att förbättra resultaten efter ö-
cellstransplantation finns därför ett ökande intresse för alternativa målorgan, vilket denna avhandling är fokuserad på.

Delarbeten

I det första arbetet studerades muskel som ett alternativt målorgan för ö-transplantation. Öar isolerades från friska möss och transplanterades sedan till bukmuskeln hos friska och diabetiska möss med eller utan polymeriserat hemoglobin (en biologisk syrgasbärrare). Målet var att studera hur stor del av de transplanterade öarna som lider av syrebrist under de första dagarna efter transplantation till muskel samt om det kan förbättras med hjälp av polymeriserat hemoglobin. Vi fann att öar som transplanteras till muskel lider av uttalad syrebrist i det akuta skedet men att det är övergående. Det har tidigare visats vid transplantation av öar till levern att det finns öar som lider av syrebrist även efter en månad. Polymeriserat hemoglobin minskade effektivt den akuta syrebristen och förbättrade dessutom de transplanterade öarnas funktion.


I det tredje arbetet studerades oment som ett potentiellt målorgan för ö-transplantation. Oment är en del av bukhinnan och själva ordet är latin och betyder ”förkläde”, vilket förklaras av dess utseende då det är fäst i mag-säcken och ”hänger” ner över tarmarna. Oment har till uppgift att skydda tarmar och bukorgan och kan begränsa infektioner och skador genom att täcka över det drabbade området. I oment finns det rikligt med kärl och ofta även inlagringar av fett. Efter transplantation av öar till oment observerades en effektiv inväxt av kärl och efter en månad var mängden kärl återställd. Även blodflödet och syresättningen i öar transplanterade till oment var jämförbar med nativa öar. Genom att använda en speciell mus-stam som saknar immunförsvar kunde humana öar transplanteras till oment och även de har en effektiv kärlnybildning och god syresättning. Efter botande transplantationer till oment hos diabetiska möss är öarnas funktion bättre jämfört med öar transplanterade till levern.

Det femte arbetet utvärderar möjligheten att kvantifiera mängden insulin-producerande celler efter transplantation till muskel med ett radioaktivt märkt spårämne, exendin-4, som är utvecklat för positronemissionstomografi (PET). Öar transplanterades i varierande mängd till bukmuskeln hos möss och sedan injicerades spårämnet och dess radioaktiva signal uppmättes i olika organ. Den totala mängden insulinproducerande celler bestämdes histologiskt med hjälp av immunohistokemiska färgningar. Vi fann att spårämnet ackumulerades i de transplanterade öarna och att den radioaktiva signalen korrelerade till mängden överlevande beta-celler.

Det sjätte arbetet utgörs av en klinisk studie där möjilikheten att bestämma mängden insulinproducerande celler i bukspottskörteln med hjälp av PET utvärderades. Spårämnet, 5-hydroxytryptofan (5-HTP), som tas upp och ackumuleras i beta-celler märktes med en svag radioaktiv signal. I studien jämfördes upptaget av 5-HTP i bukspottskörteln hos personer med typ 1 diabetes med det hos friska personer. Hos personerna med typ 1 diabetes fanns inte någon mätbar insulinproduktion och därmed troligen inga, eller endast ett fåtal, kvarvarande beta-celler. Vid undersökningar med PET fann vi att upptaget av 5-HTP hos patienter med typ 1 diabetes var 66 % lägre jämfört med friska personerna vilket faktiskt motsvarar den genomsnittliga mängden beta-celler i Langerhanska öar.

Slutsats


PET i kombination med exendin-4 eller 5-HTP som spårämne är en lovande metod för kvantifiering av transplanterade respektive nativa beta-
celler. Etableringen av en metod för att bestämma mängden beta-celler kan bli ett viktigt verktyg vid uppföljningen efter ö-transplantation samt att ha stor betydelse för att öka vår förståelse för utvecklingen och uppkomsten av diabetes.
The majority of the work presented in this thesis was carried out at the department of Medical Cell Biology, Uppsala University, Sweden. Obviously a thesis is not truly the work of one person and I would therefore like to extend my sincere gratitude to all the people involved in the work presented and especially to all my co-authors.

My supervisor Professor Per-Ola Carlsson for being a great supervisor, a source of inspiration and a role-model both as a scientist and clinician (but not so much as a driver and car-owner 😊).

My co-supervisor Associate professor Joey Lau Börjesson for your tireless help, for teaching me everything I know about laboratory techniques and for always taking the time to discuss my projects.

My co-supervisor Professor Mia Phillipson for encouragement, scientific discussions and for asking me tough questions which forced me to read much more.

Professor Leif Jansson for taking me on as a project student and for sharing your vast knowledge and interest in physiology and especially the marvelous islets of Langerhans.

Professor Arne Andersson for great discussions, guidance and very much appreciated/needed help with proof-reading. And for being an excellent room-mate 😊

All senior Professors at the department and especially Gunilla Westermark, Nils Welsh, Michael Welsh, Ulf Eriksson, Stellan Sandler, Erik Gylfé and Anders Tengholm for numerous suggestions, discussion and for your excellent, but sometimes dreaded, questions during seminars.

Present and former Head of the Department Professor Nils Welsh and Professor Erik Gylfé.

My Quach for all the invaluable help and for being an expert at surgery and sushi 😊
Lisbeth Sagulin for all the invaluable technical assistance and for all the favors, I might ask you for just a few more 😊

Petra Franzén for helping me greatly with everything regarding genes and PCR and Zhanchun Li for always being positive and an expert in immunohistochemistry.

Monica Sandberg for all the help and valuable discussion.

All members of POs research group for the discussions and interaction despite the lack of group meetings 😊

All fellow PhD-students at the department for contributing to the nice atmosphere and the many laughs shared in the coffee-room and especially at all the “julgransplundringar”. Especially thanks to Kailash Singh for great discussions and collaborations which I look forward to continuing without the distraction of my thesis 😊

All former PhD-students at the department for all the help and inspiration when I started, especially Gustaf Christoffersson and Marie Oskarsson.

A thousand thanks to the amazing Ing-Britt Hallgren, Astrid Nordin, Birgitta Bodin and Eva Törnelius for not only helping me with a great deal of things but especially for pioneering the islet research at the Department of Medical Cell Biology for more than 50 years!

The excellent research nurses Violeta Armijo Del Valle, Rebecka Hilmius and Karin Kjellström for all your enthusiasm and tireless efforts in all the clinical trials.

Professor Olle Korsgren, Department of Immunology, Genetics and Pathology, for all the great discussions and collaborations which have truly enriched my time as a PhD-student.

Associate Professor Olof Eriksson for being a good friend and for all the scientific collaborations which I hope to continue for a long time!

Professor Andre F. Palmer and PhD Uddyalok Banerjee at Ohio State University for synthesizing polymerized hemoglobin.

Professor Lena Kjellén and Inger Eriksson for giving me the opportunity to participate in your research as a teenager which truly consolidated my interest in science.
All the talented and helpful staff at the animal facility for expert care of my animals.

All of the talented staff at the clinical PET-center, and especially **Mimmi Lidholm**, for your dedication to making all the projects involving PET possible.

All the friends I have made during my time as a PhD-student for the informal scientific discussions and for all the times not circulated around research, especially **David Berglund, Fanny Fredriksson, Johan Virhammar** and **Johan Staaf**.

**Caffeine** not only for your positive effects on islets (219) but especially for your positive effects on diabetes researchers and your much appreciated help with my thesis 😊

My uncle Professor **Jonas Hugosson** for encouraging me to study medicine.

My late grandfather **Kurt Hugosson** who always truly believed in and encouraged me. You were a remarkable man and continue to be a source of many laughs and inspiration!

My father **Anders Espes** and mother **Carin Hugosson Espes** for all the encouragement, support and for always believing in me. My grandmothers **Ella-Britt Espes** and **Mona Lothigius** for inspiration, support, great talks and many laughs. My brother **Emil Espes** and sister **Frida Espes** for being great siblings and friends.

My parents in law **Conny Eriksson** and **Carina Helsén Eriksson** for all the love and encouragement.

My brother in law **Johan Eriksson** and **Madeleine Danielsson** for their support, and **Ella** and **Alice** for truly taking my mind of research and at the same time making me so much more focused in my efforts.

My wife, **Katrin Espes**, for being so encouraging and supporting in every aspect of my life and especially during all the time I spent working on my thesis. There are not words to describe the love that I have for you!
The work presented in this thesis would not have been made possible if not for the funding from:

- The Swedish Research Council
- The Swedish Diabetes Foundation
- The Swedish Juvenile Diabetes Fund
- Diabetes Wellness Sverige
- JDRF
- Torsten Söderberg Foundation
- Novo Nordisk Foundation
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Acta Universitatis Upsaliensis

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Editor: The Dean of the Faculty of Medicine

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