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# Chemical Oxygenation of Pancreatic Tissue Prior to Islet Isolation and Transplantation

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## 1. Introduction

Islet transplantation has been established as a promising treatment for patients suffering from life-threatening hypoglycemic episodes (Shapiro et al., 2000, Shapiro et al., 2006). Apart from the achievement of insulin independence, islet transplantation has been shown to improve metabolic control (Meyer et al., 1998), renal and cardiovascular function (Fiorina et al., 2003a, Fiorina et al., 2003b) and to ameliorate the progression of diabetic complications such as retinopathy (Thompson et al., 2008, Warnock et al., 2008). With regard to long-term posttransplant outcome islet transplantation alone has now reached equivalent function rates when compared to pancreas transplantation alone (Bellin et al., 2008, Vantyghem et al., 2009, Maffi et al., 2011). However, the broad application of this treatment on diabetic patients is limited with respect to the number of suitable islet preparations required to induce long-term insulin independence in recipients of islet allografts (Guignard et al., 2004, Kempf et al., 2005).

In order to reduce costs and save donor resources by increasing the efficacy of the technically challenging procedure for human islet isolation, several collaborative networks have been established between geographically distant donor centers and a core facility long-term experienced in producing high-quality islets (Brunicardi et al., 1995, Rydgard et al., 2001, Berney et al., 2004, Goss et al., 2004). Nevertheless, organ shipment between centers is frequently associated with prolonged ischemia. Since approximately 10% of the normal metabolic activity is still operative in ischemic tissue stored at 4°C, hypothermic organ perfusion and subsequent immersion in various preservation solutions such as University of Wisconsin solution (UWS) or Histidine-Tryptophan-Ketoglutarate (HTK) do not completely prevent irreversible pancreas injury once a critical period of cold ischemia is exceeded (Lakey et al., 1995). This can be explained by the specific preference of islets for the respiratory pathway of glucose breakdown providing more than 95% of the total islet ATP production (Erecinska et al., 1992) if an adequate supply oxygen for cellular energy generation is provided (Hellman et al., 1975, Malaisse et al., 1988, Sekine et al., 1994, Tamarit-Rodriguez et al., 1998). Although islets represent only 1% of the pancreatic tissue, they receive more than 12% of the total pancreatic blood flow (Lifson et al., 1980, Jansson and Hellerstrom, 1983). As a consequence, any ischemic situation has dramatic effects on the energy generation of islets (Hellman et al., 1969) which affects energy-sensitive mechanisms

such as the sodium-potassium ATPase. Since this enzyme is essentially required to counteract the intracellular osmotic pressure derived from intracellular proteins and impermeable anions, any suppression results in significant cell swelling (Belzer and Southard, 1988). Nevertheless, among other organs the exocrine pancreas has the capability to produce low amounts of energy during ischemia utilizing the Pasteur effect i.e. the increased anaerobic generation of ATP via glycolysis (Hellman et al., 1975). The increased production of lactate causes intracellular acidosis which is one of the main potential triggers of the premature intracellular auto-activation of trypsinogen in acinar cells (Gorelick and Otani, 1999). Another key factor for activation of autolytic processes within the pancreas is the increase of intracellular calcium levels related to the suppression of calcium ATPases by hypoxic conditions. These enzymes are localized in the plasma membrane as well as in mitochondrial and endoplasmic membranes and maintain low cytoplasmic calcium concentrations that are essentially required for intracellular signaling (Arnould et al., 1992, Raraty et al., 1999). Since 90% of the proteins that are synthesized by acinar cells are digestive enzymes, also short periods of ischemia provide ideal conditions to trigger autolytic processes in the pancreas (Steer, 1999, Piton et al., 2010). Although the Pasteur effect does not seem to be relevant for islets (Sekine et al., 1994, Tamarit-Rodriguez et al., 1998), it is quite likely that extensive autolysis affects the functional and morphological integrity of adjacent islets (Tanioka et al., 1997a). Analysis of more than 150 diabetic recipients revealed that achievement of insulin independence after islet allotransplantation correlates with short cold ischemia times (CITR, 2009).

Several approaches have been performed to improve oxygen supply of retrieved organs during cold storage: (1) Continuous hypothermic machine perfusion (HMP) (Wight et al., 2003, Schold et al., 2005, Treckmann et al., 2011). Modifications of this preservation procedure include the use of continuously oxygenated perfusion media as demonstrated in different animal models (Manekeller et al., 2005, Maathuis et al., 2007, Stegemann et al., 2009). First attempts to use HMP for pancreas preservation prior to islet isolations were successfully performed in the pig pancreas (Taylor et al., 2010). Nevertheless, with regard to its high costs and its low availability for a maximum of 20% of transplanted organs in the United States (Maathuis et al., 2007) or less than 4% worldwide (Opelz and Dohler, 2007), the relevance of hypothermic machine perfusion is still under discussion (Watson et al., 2010). (2) Persufflation. The continuous gaseous supply of humidified oxygen via the vessels of explanted kidneys or livers represents another approach to improve oxidative energy metabolism during ischemia (Minor and Isselhard, 1996, Pegg et al., 1989, Minor et al., 2002). Recently, a pilot study was performed to utilize persufflation to rescue marginal donor livers for transplantation into patients (Treckmann et al., 2008). Although the principles of retrograde persufflation has been established in 1972 (Isselhard et al., 1972) first pilot trials in human and porcine pancreases have been reported just recently (Scott et al., 2010). However, the concerns that have been raised regarding the logistical disadvantages of hypothermic machine perfusion and costs related to complex equipment for controlled oxygen generation and additional personnel needed for continuous supervision seems also to be valid for persufflation (Feng, 2010). (3) The incubation of organs in oxygen-precharged perfluorocarbons. One member of this chemical group, perfluorodecalin, has been extensively investigated in pancreases obtained from different species. (4) The perfusion of retrieved organs utilizing oxygen-charged emulsions. In the present chapter we focus primarily on the latter two items.

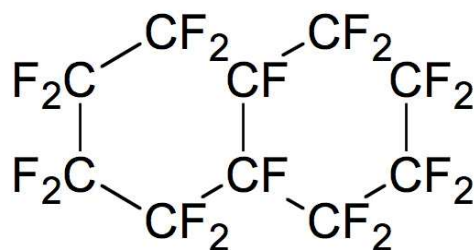


Fig. 1. Chemical structure of perfluorodecalin.

## 2. Tissue oxygenation prior to solid organ transplantation

Perfluorodecalin (PFD) is a hydrocarbon of bicyclic structure in which all hydrogen atoms are replaced by fluorine atoms (Fig. 1). As a consequence of the high number of carbon-fluorine bonds the specific gravity of PFD is close to 2.0 g/mL (Wong and Lois, 2000). For its use as oxygen carrier it is important that this substance is characterized by a high oxygen dissolving capacity of 45 – 50% (vol./vol.) but at the same time it has a negligible binding constant for oxygen which allows to release oxygen more efficiently into tissue than hemoglobin (Matsumoto and Kuroda, 2002).

First attempts to use PFD for pancreas oxygenation during cold storage prior to autotransplantation were performed in 1988 utilizing canine pancreases (Kuroda et al., 1988). This procedure was established as two-layer method (TLM) because tissue is generally floating on the high gravity PFD and has to be covered by organ preservation solution such as Euro-Collins (Kuroda et al., 1990b) or UWS (Kuroda et al., 1992b) forming a second layer on the immiscible PFD (Fig. 2). During cold storage the oxygen carrier is continuously gased with 95% oxygen and 5% carbondioxide CO<sub>2</sub> from the bottom of the storage container which results in a partial oxygen pressure (pO<sub>2</sub>) of approximately 600 mm Hg (Matsumoto and Kuroda, 2002). As shown in canine (Kuroda et al., 1992a, Matsumoto et al., 1996b) and human pancreases (Kuroda et al., 1992b) this supranormal pO<sub>2</sub> seems to be effective to stimulate the mitochondrial ATP synthesis within the cold-stored pancreatic tissue. The efficiency of this process can be further increased when adenosine is supplied as ATP precursors (Kuroda et al., 1994a, Hiraoka et al., 1994). However, an ongoing ATP synthesis seems to be important not only to preserve pancreatic endocrine function during prolonged cold storage (Kuroda et al., 1991) but also for recovery of ischemically damaged pancreases (Kuroda et al., 1993b).

Inspite of the amazing results that were obtained after autotransplantation of canine pancreases long-term stored for up to 72 and 96 hours (Kawamura et al., 1989, Kuroda et al., 1992c) or exposed to 90 min of warm ischemia time (Kuroda et al., 1993a) only one study to perform TLM prior to clinical pancreas transplantation has been reported so far. Although an improvement in tissue quality and posttransplant function was observed, these differences were not significant when compared to storage in UWS (Matsumoto et al., 2000).

The failure to establish TLM for clinical pancreas transplantation may be related to its complex arrangement which complicates transportation of retrieved organs.

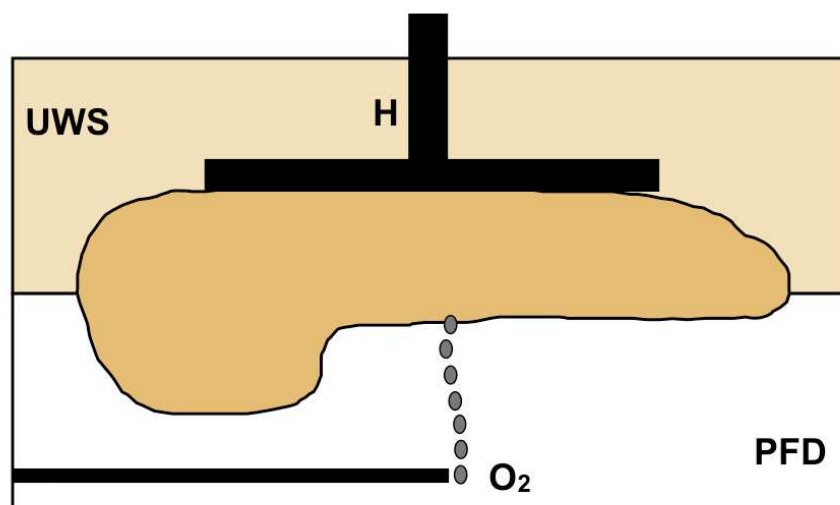


Fig. 2. Pancreas oxygenation utilizing the TLM. The pancreas is floating at the interface between University of Wisconsin solution (UWS) and perfluorodecalin (PFD) and continuously gassed with oxygen ( $O_2$ ). The high gravity of PFD is counteracted utilizing a holder (H) to press the pancreas under the surface of the oxygen carrier.

The practicability of TLM was therefore doubted soon after its introduction. In order to simplify TLM, experiments were performed to assess the efficiency of oxygenation when ischemic pancreases are completely immersed in PFD. It was demonstrated that canine pancreases can be successfully preserved for 48 hours utilizing a one-layer method (OLM) by removing the top-layer consisting of Euro-Collins or UWS (Kuroda et al., 1990a). However, when the cold storage time was prolonged to 96 hours, efficiency of OLM appeared to be inferior compared to TLM (Kin et al., 1993). In contrast, comparative experiments in rats revealed that pancreas preservation for 48 or 72 hours is significantly improved using OLM in comparison to TLM (Urushihara et al., 1992, Urushihara et al., 1994). The principle of OLM was revived some years later in order to improve pig pancreas preservation prior to islet isolation. In agreement with Urushihara's studies it was found that islet tissue isolated after prolonged cold storage is characterized by a significantly higher potency in terms of insulin secretory capacity, membrane integrity, mitochondrial activity, ATP generation and posttransplant function in diabetic nude mice when the organ is completely immersed in PFD and compared to TLM (Brandhorst et al., 2005).

The idea to immerse organs completely in PFD was picked up to improve preservation of cavernous organs after flushing the cavities with UWS. Using this procedure the small intestine retrieved from rats (Kuroda et al., 1996b, Tsujimura et al., 2004b) or dogs (Tsujimura et al., 2002b, Fujino et al., 2003) could be successfully preserved for 24 to 48 hours. In addition, this technique provided also excellent survival rates of 80% after heterotopic transplantation of UW-perfused rat hearts after cold storage for 48 hours in oxygenated PFD (Kuroda et al., 1995). Remarkably, even higher survival rates in recipients were observed after 72 hours of cold ischemia utilizing a gas mixture composed of 90% oxygen and 10%  $CO_2$ . When 100% oxygen was used for preservation complete graft failure was observed (Yoshida et al., 2008). The reason for this discrepancy are unknown but it was



speculated that a certain percentage of CO<sub>2</sub> decreases the metabolism of cells beyond the level that can be expected according to the storage temperature (Mitsuda et al., 1987). The period of preservation could successfully be extended to 96 hours when the hearts were continuously perfused during oxygenation with Krebs-Henseleit buffer. In the case that the hearts were continuously perfused for 96 hours with UWS the success rate decreased from 80 to 0% (Hatayama et al., 2009).

Only a few reports have been published so far utilizing conventional TLM for oxygenation of organs other than the pancreas. The suitability of this technique for solid organ preservation was recently demonstrated in syngeneic rat kidneys assessing graft posttransplant survival and apoptosis after 24 hours of cold storage in comparison to UWS. It was observed that posttransplant outcome with respect to one-month survival and creatinine levels was significantly improved compared to UWS storage. In addition, histological tissue damage and frequency of apoptosis were significantly reduced after TLM storage (Marada et al., 2010). In contrast, attempts to preserve porcine kidneys utilizing TLM resulted in increased inflammation, tissue injury and reduced renal function which raised the question whether TLM is suitable for organs from species larger than rodents (Hosgood and Nicholson, 2010). Moreover, experiments to successfully preserve rat lungs during 6 hour-storage by means of TLM failed to demonstrate significant improvement of graft survival compared to cold storage in Ringer's lactate, UWS or Celsior. Lungs transplanted after TLM were characterized by more infiltrates of inflammatory cells compared to the other experimental groups. Surprisingly, TLM-stored lungs continuously oxygenated during 6 hour-storage had the lowest oxygen saturation of all media assessed (Liu et al., 2007). It can be speculated that the latter finding was made because the organs were not fixed at the UWS-PFD interface and were rather floating in UWS. In agreement, other approaches to preserve functional integrity of rat livers during extensively prolonged cold storage were also not successful when compared to cold storage in modified UWS (Sumimoto et al., 1990). The authors of this study assumed that in addition to the surface-to-volume ratio also the texture and structure of an organ defines whether inner layers of the tissue can efficiently be supplied with oxygen or not.

### 3. Pancreas oxygenation for subsequent islet transplantation

First approaches to isolate islets after storage in oxygenated PFD were performed in the dog pancreas. Immediately after retrieval, pancreases were placed on continuously oxygenated PFD until islet isolation was initiated after 3 or 24 hours. It was shown that significantly more islets can be isolated utilizing TLM during prolonged cold storage compared to UWS. However, these experiments are difficult to compare with subsequent studies in human pancreases, since retrieved canine pancreases were not perfused with organ preservation solution prior to cold storage (Tanioka et al., 1997b). The setting for first studies in human pancreases was different to subsequent ones because of logistical reasons. In these experiments retrieved pancreases were shipped in UWS to the isolation facility, dissected to remove excessive fat and connective tissue and either immediately processed or oxygenated by TLM for an additional period of cold storage. Nevertheless, in spite of prolonged cold ischemia time significantly more islets could be isolated and recovered after culture compared to storage in UWS (Matsumoto et al., 2002a). These promising results could be confirmed in a similar setting by achieving a significantly

higher islet transplantation rate of 71% in pancreases that were oxygenated for additionally 3 hours compared to UWS storage alone (36%) (Tsujimura et al., 2002a). An even more impressive improvement of the success rate from 11% to 53% was obtained in TLM-oxygenated pancreases from marginal donors older than 50 years (Ricordi et al., 2003). However, it should be stressed that in 80% of the organs used in the latter study oxygenation started immediately after retrieval utilizing oxygen-precharged PFD for oxygen supply. In a number of studies static TLM was implemented as routine procedure for oxygenation of human pancreases during shipment and cold storage prior to clinical islet isolation and transplantation (Hering et al., 2004, Hering et al., 2005, Lee et al., 2004, Goss et al., 2004).

Remarkably, in spite of exposing the tissue to supranormal  $pO_2$ , the level of reactive oxygen species (ROS) and their products is significantly lower in TLM-stored pancreases compared to UWS-preserved organs (Salehi et al., 2006). One explanation for this phenomenon is that TLM re-initiates production of ATP in mitochondrial pathways by providing sufficient oxygen levels. One can speculate that maintaining the metabolic activity of mitochondria within a certain range seems to be efficient to prevent excessive generation of ROS in mitochondria as main subcellular producers of ROS (Li et al., 2008, Tsujimura et al., 2004a) and to inhibit mitochondrial pathways of apoptotic cell death (Matsuda et al., 2003).

Beside this important aspect, the main function of mitochondria for cellular metabolism is the production of energy. The relevance of this subcellular structures for islet metabolism is demonstrated by an ATP production that is 17-fold higher compared to the glycolytic metabolism of glucose (Erecinska et al., 1992). The Krebs cycle contributes to the highest extent to the ATP synthesis in islets but is more susceptible toward hypothermia than the cytosolic pathway of non-oxidative glucose metabolism (Escobar et al., 1990). It could be demonstrated that an increase of the storage temperature to 20°C allows for significant reduction of the oxygenation time required to resuscitate canine pancreases after exposure to prolonged warm ischemia (Kuroda et al., 1996a, Matsumoto et al., 1996a). This modification bears the potential of increased flexibility in the logistics of pancreas procurement and subsequent islet isolation by facilitating short-term oxygenation after pancreas arrival in the isolation facility prior to initiating the isolation procedure. Experiments in pig pancreases revealed that determinants of successful islet isolation such as islet yield, viability, and morphological integrity were not reduced compared to non-stored pancreases when the oxygenation temperature was increased to 20°C (Iken et al., 2009). Nevertheless, in spite of an ATP generation that was enormously increased when compared to freshly isolated islets or to oxygenation at 4°C, islets isolated after oxygenation at 20°C were characterized by reduced *in vitro* function and posttransplant outcome after transplantation into diabetic nude mice (Fig. 3).

These findings are in conflict with the widely accepted hypothesis that the pancreatic ATP content correlates with posttransplant graft function (Kuroda et al., 1991, Kuroda et al., 1992a, Kuroda et al., 1993b). They rather suggest that a temperature-stimulated ATP production does neither reflect tissue viability nor predict posttransplant outcome. In contrast, the relevance of an ongoing ATP production for the recovery of pancreatic tissue predamaged by significant warm ischemia was demonstrated in canine pancreas autotransplantation (Kuroda et al., 1993a, Kuroda et al., 1993b, Kuroda et al., 1994a).

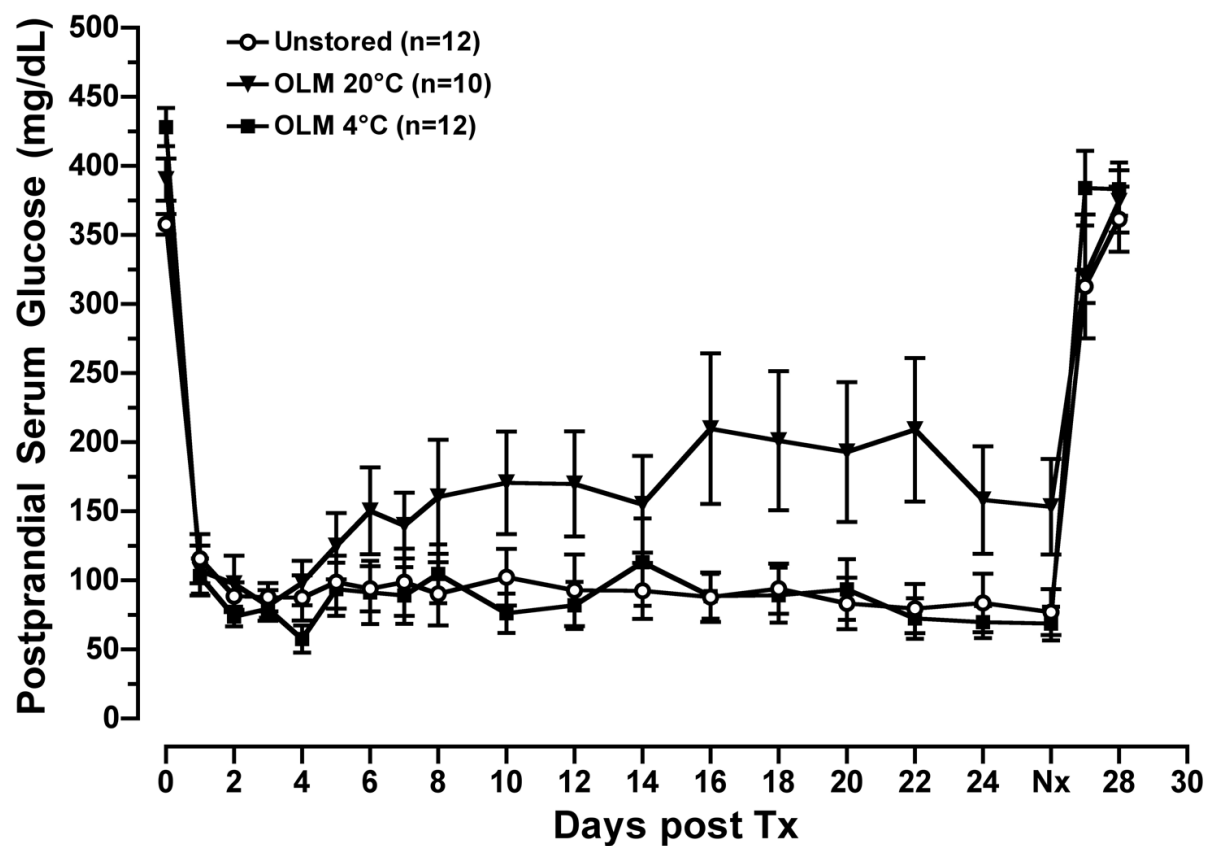


Fig. 3. Non-fasting serum glucose levels in streptozotocin-treated (240 mg/kg) diabetic NMRI nude mice after subcapsular transplantation of pig islets isolated from pancreases (n=3) either immediately procured (unstored, open circles) or subjected to prior oxygenation utilizing the one-layer method (OLM) at 20°C (OLM 20°C, filled triangles) or 4°C (OLM 4°C, filled squares). Graft removal through nephrectomy (Nx) was performed as indicated at day 26 posttransplant.

In agreement, assessment of samples retrieved from cancer patients revealed that TLM increases the ATP content in ischemically predamaged pancreatic tissue (Kuroda et al., 1994b). Islet isolation was not performed in this study. Similarly, a prospective study in adult pigs clearly demonstrated as well that oxygenated PFD can significantly increase the ATP content in islets isolated from pancreases that were pre-exposed to 30 min of warm ischemia. In this study continuous oxygenation for 3 hours did not prevent ischemia-induced deterioration of islet yield and posttransplant function in diabetic nude mice although the ATP content measured after warm ischemia and PFD storage reached the same level as in functioning islets isolated after oxygenation but without warm ischemia time (Brandhorst et al., 2006). This finding is in contradiction with another study in isolated pig islets demonstrating a strong positive correlation between intraislet ATP content and posttransplant function in diabetic nude mice (Kim et al., 2009). Remarkably, the ATP content of the successful (PFD, no warm ischemia) and the failed group (PFD + warm ischemia), as measured in our study, was in a similar range that defined transplant success in Kim’s study. Another approach in isolated pig islets established the ATP-to-



ADP ratio as reliable predictor for posttransplant function using again diabetic nude mice as recipients (Goto et al., 2006). However, another contribution to this ongoing discussion about the relevance of ATP determination in isolated islets clearly preferred the ATP-to-DNA ratio compared to the ATP-to-ADP ratio because of the stability of DNA that favours the preciseness and linearity of this assay (Suszynski et al., 2008). However, as long as no more data are available regarding ischemia, islet ATP production and posttransplant outcome, it can be assumed that oxygen-stimulated ATP synthesis in ischemic pancreases from large mammals does not improve posttransplant islet function.

The scepticisms toward the efficiency of PFD utilized as oxygen carrier for pancreases from large mammals has been additionally supported by two large scale studies which includes more than 350 islet isolations. In contrast to previous experiments that were performed in relatively small numbers of pancreases, these studies clearly revealed that pancreas storage in precharged PFD is unsufficient to increase islet yield, insulin secretory capacity and posttransplant function in recipients when compared to organ storage in UWS (Kin et al., 2006a, Caballero-Corbalan et al., 2007). Several reasons can be discussed for this observation. One important difference that exists between the larger trials and previously published smaller studies concerns the method of oxygenation used. While in the smaller trials pancreases had been subjected to continuous oxygenation for a short period of approximately 3 hours subsequent to cold storage (Tsujimura et al., 2002a, Tsujimura et al., 2004a, Salehi et al., 2006), the large scale trials utilized oxygen-precharged PFD for the entire period of cold ischemia. However, experiments in rats clearly suggested that oxygen-precharged PFD is equivalent to continuously oxygenated PFD with regard to pancreas ATP concentration and isolated islet yield after 24 hours of cold storage (Hiraoka et al., 2001). These findings were confirmed in human pancreases since no difference was observed regarding islet isolation outcome between the static and original TLM (Matsumoto et al., 2002b).

A further technical detail that most likely affects the outcome of pancreas oxygenation is the trimming of the pancreas before incubation in PFD in order to remove non-parenchymatic tissue interfering with oxygen penetration into the pancreatic core. As reported, this was the case for the studies from the Edmonton group (Tsujimura et al., 2002a, Tsujimura et al., 2004a, Salehi et al., 2006, Kin et al., 2006a) but not for the study from the Nordic Network (Caballero-Corbalan et al., 2007). This is of particular relevance when pancreases from obese donors are recovered. These organs are mostly embedded in obstructive quantities of fat aggravating oxygenation of the tissue (Brandhorst et al., 1995). Apart from these technical considerations it has been doubted that it is physically possible at all to efficiently supply oxygen to a pancreas retrieved from large mammals.  $pO_2$  measurements in different species indicated that the size of a pancreas correlates inversely with the proportion of tissue that can be sufficiently supplied with oxygen following a gradient from the outer surface to the core of the organ. While approximately 80% of a rat pancreas are supplied with oxygen utilizing TLM, this proportion is less than 20% in an averaged-sized pig or human pancreas preserved by the same procedure (Papapoulos et al., 2005, Avgoustiniatos et al., 2006). Since the pancreas volume correlates positively with either body height or body surface area (Goda et al., 2001, de la Grandmaison et al., 2001, Kin et al., 2006b), it can not be excluded that the selection of slim donors has a significant effect on efficiency of pancreas oxygenation during cold storage (Salehi et al., 2006).

#### 4. Alternative oxygen carriers for organ oxygenation

Widely accepted data from canine pancreas autotransplantation suggest a positive correlation between pancreas oxygenation, tissue  $pO_2$ , ATP generation and posttransplant graft function (Matsumoto et al., 1996b) but are partially in conflict with findings in human and porcine pancreas preservation as discussed above. The contradiction may be explained, on the one hand, by species-dependent differences with regard to pancreas size, firmness and texture. On the other hand, it has to be discussed whether the hydrophobic and lipophobic character of PFD prevents oxygen penetration into the pancreatic core at all. The solubility of PFD in native olive oil, a parameter for lipophilicity, is only 1.1%. Amphiphilic oxygen carriers, such as perfluorohexyloctan (F6H8), a semifluorinated alkane with a similar oxygen-dissolving capacity as PFD, reach a solubility of 23.4% (Hoerauf et al., 2001). The low lipophobic character of F6H8 can be attributed to the high number of lipophilic CH-groups (Fig. 4), which are completely absent in PFD (Fig. 1). As a result, F6H8 has a much lower specific gravity (1.35 g/cm<sup>3</sup>) compared to PFD (1.93 g/cm<sup>3</sup>). We hypothesized that oxygenation of ischemic human pancreatic tissue can significantly be improved when lipophilic oxygen carriers are used for shipment from the donor to the recipient center.

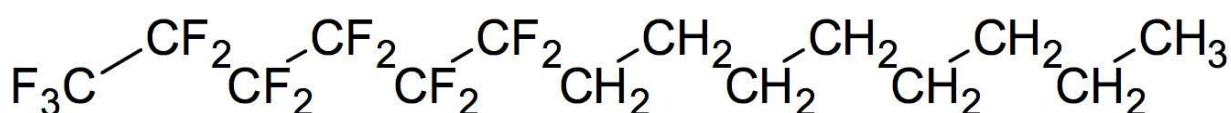


Fig. 4. Chemical structure of perfluorohexyloctan (F6H8).

Initial experiments in rats clearly demonstrated that pancreas oxygenation utilizing the lipophilic compound F6H8 for 24 hours of cold storage significantly improved islet isolation outcome in terms of yield, viability and functional integrity of isolated islets as well as transplant function in diabetic nude mice when compared to PFD (Brandhorst et al., 2009). In agreement with the postulation of Avgoustiniatos (Avgoustiniatos et al., 2006), small organs like rat pancreases can efficiently be provided with oxygen regardless of the chemical characteristics of the oxygen carrier used. Utilizing Clark-type microelectrodes probes we found that the  $pO_2$  levels in oxygenated rat pancreases exceeded that of non-stored rat pancreatic tissue by four-fold. Nevertheless, inspite of the extremely increased intrapancreatic  $pO_2$ , the ATP content in oxygenated rat pancreases was unexpectedly low when compared to unstored organs. The relatively low ATP production may be explained by the observation that the capacity of oxygenated PFD to stimulate oxidative ATP synthesis in rat pancreases is limited to approximately 12 hours (Scott et al., 2008). It can further be speculated whether a supplementation of fuels such as glucose or pyruvate, which are missing in UWS or HTK, would prevent this energy exhaustion in long-term preserved tissue.

In contrast to the observations made in rats, we demonstrated in porcine and human pancreases that the amphiphilic compound F6H8 is superior for the oxygen supply of pancreatic tissue compared to inert PFD (Fig. 5). To discuss the relevance of these findings for preservation of islet tissue prior to human islet isolation, we have to refer to the extensive work of Carlsson et al. evaluating the  $pO_2$  in native and transplanted islet tissue from rats. According to the findings of this group, the  $pO_2$  in human pancreases oxygenated by means of F6H8 reached only 20 – 25% of native vascularized pancreatic tissue provided

that human and rat tissue are similar in blood supply and oxygen consumption (Carlsson et al., 1998, Carlsson et al., 2000). Nevertheless, transplanted human islets can survive for months under hypoxic conditions that correspond to a  $pO_2$  of approximately 5 mm Hg or less (Carlsson et al., 2002). This would mean that the level of oxygenation provided by F6H8 is sufficient to support islet survival during prolonged pancreas cold storage.

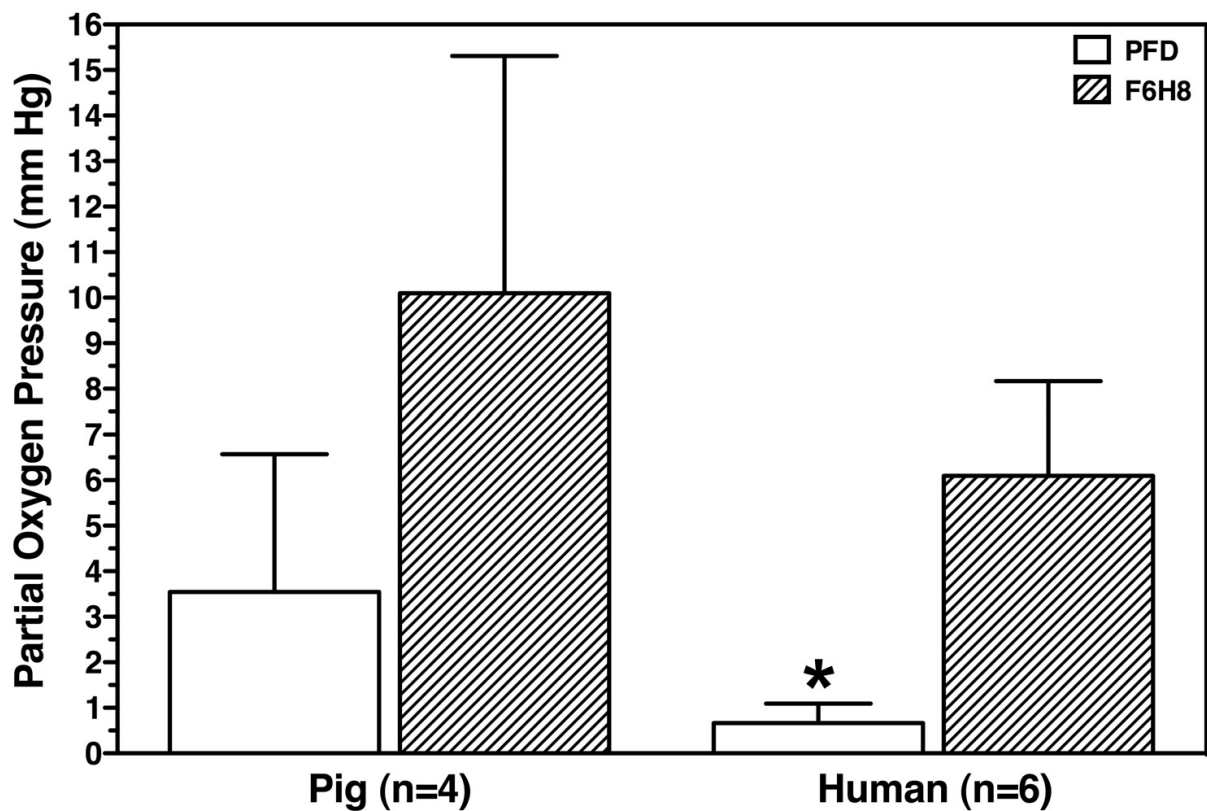


Fig. 5. Intrapaneatic partial oxygen pressure measured in porcine and human pancreases after static oxygenation in PFD (blank bars) or F6H8 (hatched bars) for 8 (pig, n=4) or 24 hours (human, n=6) of cold storage. Pancreas oxygenation was performed utilizing the one-layer method. \*P<0.05 by Mann-Whitney test as indicated (mean  $\pm$  SEM).

The significance of this assumption for human islet isolation outcome was confirmed in human research grade pancreases processed for subsequent islet isolation after storage in oxygen-precharged F6H8 or PFD for at least 24 hours. Compared with PFD, F6H8 significantly increased islet yield, islet cell survival after culture, insulin secretory capacity and posttransplant function in diabetic nude mice (Brandhorst et al., 2010). In agreement, studies in guinea pigs demonstrated improved quality of hearts that were stored for 6 hours of cold ischemia in oxygenated F6H10, another compound belonging to the group of semifluorinated alkanes (Isaka et al., 2005).

Nevertheless, larger organs such as liver or kidney may require vascular supply of oxygen for efficient oxygenation of the entire tissue volume (Hosgood and Nicholson, 2010). As discussed above, the efforts and costs related to continuous perfusion of donor organs with oxygenated preservation solutions are currently limiting the broad implementation of this technique (Opelz and Dohler, 2007). Emulsions made from perfluorocarbon-

derived oxygen carriers represent an alternative that is easy to apply during regular organ procurement. We hypothesized that the vascular flush as routinely performed during organ procurement can cover the requirements for both effective tissue preservation and oxygen supply of large organs, if organ preservation solutions combine a high oxygen-dissolving capacity with characteristics of HTK or UWS, vital to prevent cell swelling and maintain tissue viability during cold storage (Belzer and Southard, 1988). However, the chemical requirements to manufacture stable emulsions including a significant percentage of oxygen carrier clearly favour the utilization of semifluorinated alkanes such as F6H8 or F6H10 which possess amphiphilic characteristics in contrast to PFD (Klar et al., 1998, Voiglio et al., 1996).

## 5. Conclusions

In summary, the prevention of ischemically induced tissue damage in retrieved donor organs during cold ischemia is still a major logistical problem that remains to be solved. This concerns particularly organs with a low ischemic tolerance like the pancreas including the islets of Langerhans. Tissue oxygenation utilizing complex techniques such as continuous machine perfusion or persufflation is more or less established but available only for a limited number of procurement centers. In contrast, the concept to provide oxygen by means of precharged perfluorocarbons for static cold storage is attractive because of its low costs and simplicity. Although the majority of findings obtained in smaller animals clearly indicate that this concept is feasible, efficient oxygenation of organs retrieved from larger species such as humans is difficult because of organ size. One solution to solve the problem of efficient oxygen supply may be provided by utilization of semifluorinated alkanes, lipophilic oxygen carriers that can penetrate into the core of ischemic organs in contrast to inert substances like PFD. Another way to overcome the hurdle to completely oxygenate larger human organs is to use vascular perfusion as routinely performed during organ procurement to administrate oxygen-precharged emulsions. For effective organ preservation these emulsions should ideally combine a high oxygen-dissolving capacity with characteristics of established organ preservation media such as HTK or UWS. Efforts are currently undertaken to evaluate the potential of this new concept in large animal models.

## 6. Acknowledgements

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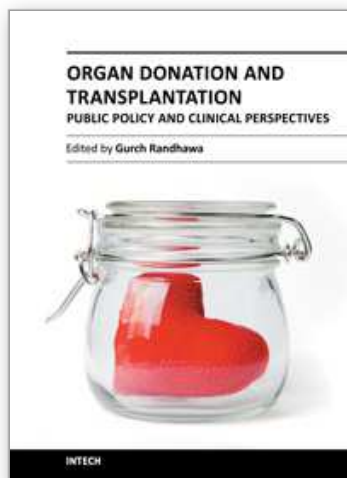
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