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Original contribution

# Pre-transplantation $^{31}$P-magnetic resonance spectroscopy for quality assessment of human pancreatic grafts – A feasibility study

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ABSTRACT

*Objective:* To investigate the feasibility of using $^{31}$P-MRS for objective non-invasive quality assessment of human pancreas grafts prior to transplantation or islet isolation.
*Materials and methods:* Pancreata from 5 human donors, 3 males and 2 females, aged 49–78 years, with body mass index (BMI) 22–31 kg/m$^2$, were included. Pancreata were perfused with histidine-tryptophan-ketoglutarate solution during procurement and stored in hypothermic condition (4 °C) for 21–44 h. During the period of hypothermic storage repeated spectra were obtained for each graft by $^{31}$P-MRS (1.5 Tesla) to measure the cold ischemia time (CIT) dependent changes of the phosphorous metabolites adenosine triphosphate (ATP), phosphomonoesters (PME), phosphodiesters (PDE) and inorganic phosphate (Pi), in the grafts. Graft temperature was measured immediately before and after MR-examination. Reference spectrum for non-viable tissue was obtained after graft exposure to room temperature.
*Results:* PME/Pi, PDE/Pi and ATP/Pi spectral intensities ratios decreased with increasing CIT, reflecting the decreased viability of the grafts. PME/Pi ratio was the most discriminatory variable at prolonged CIT. $^{31}$P-MRS could be performed without significantly increasing graft temperature.
*Conclusions:* $^{31}$P-MRS may provide quantitative parameters for evaluating graft viability ex vivo, and is a promising tool for objective non-invasive assessment of the quality of human pancreas grafts prior to transplantation or islet isolation.



## 1. Introduction

Transplantation of pancreas or islets of Langerhans enables diabetic patients to achieve euglycaemia independently of exogenous insulin administration and is indicated in patients who have or are at high risk of severe secondary complications of diabetes and in patients with disabling or life-threatening hypoglycaemic unawareness. The typical graft recipient has type 1 diabetes with renal failure [1]. With the shortage of donors for pancreas transplantation there is a critical need to optimally use all available grafts. However, there is currently an underutilization of pancreatic grafts, largely due to the strict criteria of what constitutes an acceptable pancreas allograft [2]. Data from Eurotransplant, an international foundation responsible for the allocation of donor organs in Austria, Belgium, Croatia, Germany, Luxembourg, the Netherlands and Slovenia, indicate that only 27% of donor pancreata are transplanted, either as whole pancreas grafts or as islet grafts [3]. In the US only 13% of deceased donors provide a pancreas that is utilized for transplantation [4]. Donor age above 45, donor body mass index (BMI) above 30 kg/m$^2$ and donation after cardiac death have been shown to increase the risk for graft failure, and donor scoring systems with criteria for suitable grafts have been set up to help select grafts for transplantation [5]. However, several transplantation centers have reported good results with extended-criteria donors, that are older [6,7], have a higher BMI [8] or cardiac death [9]. There are no reliable donor factors that can accurately predict post-transplantation outcome. To date the most important selection criterion to identify suitable pancreatic allograft remains the surgeon's own assessment of organ quality during procurement [5], which is biased by individual experience and personal skills. A method for reliable objective non-invasive pre-transplantation assessment of graft quality is desirable. Such a method could potentially increase the number of successful transplantations, by optimizing graft selection and enabling enhanced utilization of the extended-criteria donor pool, without increasing the risk of post-transplantation complications and graft failure.

*Abbreviations:* BMI, body mass index; HTK, histidine-tryptophan-ketoglutarate; CIT, cold ischemia time; ATP, adenosine triphosphate; PME, phosphomonoesters; PDE, phosphodiesters; Pi, inorganic phosphate; ISIS, image-selected in vivo spectroscopy; PC, phosphocholine; PE, phosphoethanolamine; GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine; PtdC, phosphatidylcholine; NADP, nicotineamide adenine dinucleotide phosphate.

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Adenosine triphosphate (ATP) plays a vital role in cellular bioenergetics and thus organ viability. $^{31}$P magnetic resonance spectroscopy ($^{31}$P-MRS) is an established method for quantifying levels of ATP, its breakdown product inorganic phosphate (Pi) and other phosphorous metabolites, such as phosphomonoesters (PME) and phosphodiesters (PDE). These are typically expressed as ratios, for example ATP/Pi. $^{31}$P-MRS has previously been applied for assessment of human graft viability and function pre- and post-transplantation in kidney, heart and liver [10–16]. Ischemic changes, transplant rejection and graft viability of pancreas has been studied using $^{31}$P-MRS in animal models [17–23]. The findings from these studies indicate that $^{31}$P-MRS might be used to assess pancreas graft viability in the clinical setting. A study exploring the feasibility of applying this technique in human pancreata is needed before it can be implemented in larger clinical testing.

Hypothermia decreases the rate at which enzymes degrade cellular components necessary for graft viability, but does not stop the process completely. With increased cold ischemia time (CIT) the organ viability is gradually decreased [24]. Static cold storage is the standard preservation method for pancreatic grafts in the clinical setting. The aim of this pilot study was to investigate the feasibility of using ex-vivo MRS for assessment of viability of human pancreas grafts prior to transplantation or islet isolation, by correlating phosphorous metabolite ratios with CIT. The hypothesis was that ATP/Pi and PME/Pi ratios would decline with increasing CIT reflecting decreased graft viability.

## 2. Materials and methods

### 2.1. Donors

Pancreata from 5 human donors were included, Table 1 describes the donor characteristics. These grafts were not intended or utilized for transplantation due to advanced donor age (49–78 years). Consent for organ donation for clinical transplantation or for utilization in research was obtained from the relatives of the deceased donors by the donors' physicians and was documented in the medical records of the deceased subjects. All pancreas grafts for research use arriving to our center between January 2013 and May 2014 were included in the study provided that histidine-tryptophan-ketoglutarate (HTK) solution had been used for perfusion and subsequent storage, and the MR-scanner was not occupied. The study was approved by the Regional Ethics Committee. During organ procurement, pancreata were perfused in-situ with HTK-solution. Post-retrieval, all pancreata were stored in a pancreas container kit (MEDCO$_{AS,}$ Årvollskogen, Norway) filled with HTK-solution under hypothermic condition (4 °C) during the following 21–44 h. Contrary to University of Wisconsin solution, HTK-solution does not contain phosphorus; hence the signals measured by $^{31}$P-MRS in our study are solely derived from pancreas graft metabolites and not the preservation solution.

### 2.2. MR examinations

Examinations were performed on a 1.5 T clinical MR scanner (Achieva, Philips Healthcare, Best, The Netherlands). During the period of hypothermic storage each graft was repeatedly examined with $^{31}$P-MRS at different time points to measure the CIT dependent changes of phosphorous metabolites in the graft. Since the pancreata were transported from other hospitals to our center the first measurement for each graft could not be performed immediately after retrieval. The first spectrum was obtained 5–13 h after perfusion start. Due to the inherent unpredictability of the access to pancreatic grafts, where grafts suitable for the study became accessible at very short notice, the time points for the subsequent measurements could not be standardized, but had to be adjusted to available time slots for MR-examinations, between pre-planned patient examinations. After the period of cold storage three pancreata were exposed to room temperature for 4–25 h and one last $^{31}$P-MRS spectrum was measured for each pancreata. These spectra served as reference for non-viable tissue. The last spectrum of two pancreata (nr 1 and 5) was measured after 23 and 21 h of cold preservation respectively. To guide placement of spectroscopic voxels $T_2$ weighted Turbo Spin Echo sequences in three orthogonal planes were obtained with the whole body coil. Phosphorous spectra were acquired using a transmit-receive quadrature head coil. Magnetic field homogeneity was adjusted automatically by iterative first-order shimming. Volume of interest (voxel) was defined by an image-selected in vivo spectroscopy (ISIS) localization sequence [25] Hyperbolic secant adiabatic pulses were used for rf-excitations. The main measurement parameters were as follows: spectral bandwidth, 1500 Hz; repetition time, 3500 ms; 1024 points (the length of FID 683 ms), and 512 acquisitions. The first FID point was sampled 0.1 ms after the last rf-pulse. Typical voxel size was $45 \times 45 \times 140$ mm$^3$ (Fig. 1). To improve the signal-to-noise ratio and the spectral resolution the ISIS sequence was combined with $^1$H broad band decoupling (WALTZ-4 modulation) and nuclear Overhauser enhancement (NOE). The NOE mix time was 2400 ms. The whole body rf-coil was used for this purpose.

During all MR examinations ice packs were placed around the container to avoid heating of the graft (Fig. 1). The effectiveness of the cooling was evaluated by measuring the temperature of the solution inside the preservation container just before and immediately after MR examination at five different time points.

### 2.3. Spectrum processing

Spectra were fitted using the AMARES algorithm [26] as implemented in the magnetic resonance user interface software package [27] and each peak fitted with a Lorenzian and the spectral intensity (the area under the curve) was calculated. The following metabolite intensities were quantified: PME (main constituents are phosphocholine (PC) and phosphoethanolamine (PE)), Pi, PDE (consisting of glycerophosphocholine (GPC) and glycerophosphoethanolamine (GPE)), and ATP (three phosphate groups γ-, α-, and β-ATP). Spectra were phased manually and fitted without previous apodization of the free induction decays. However, for presentation purpose, a Lorentzian apodization corresponding to 2 Hz line broadening was applied. Prior knowledge for spectrum processing was obtained by fitting the well-resolved pancreas spectra and by using data from the literature. Following

**Table 1**
Gender, age and body mass index of the donors.

| Donor nr | Sex | Age (y) | BMI (kg/m$^2$) |
|---|---|---|---|
| 1 | F | 62 | 29.4 |
| 2 | M | 68 | 24.0 |
| 3 | F | 63 | 30.9 |
| 4 | M | 78 | 22.2 |
| 5 | M | 49 | 22.3 |

**Fig. 1.** Typical voxel position for $^{31}$P-MRS in the transversal (left) and sagittal (right) plane. Ice packs surround the graft container to enable continuous cold preservation.