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# Magnetic Resonance Spectroscopy (MRS) in Kidney Transplantation: Interest and Perspectives

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## 1. Introduction

Currently, in biology, Magnetic Resonance Spectroscopy (MRS) is widely used in metabonomics for diagnosis and prognostication in a wide range of studies from brain (Blasco et al., 2010) to leg (Borel et al., 2009) pathologies. Between both, the topic of interest in this section: the kidney. We will especially focus on kidney transplantation. Indeed, in France as well as in Europe, the number of patients awaiting transplant is still rising while the number of transplantations performed remains stable and even decreases (Figure 1.).

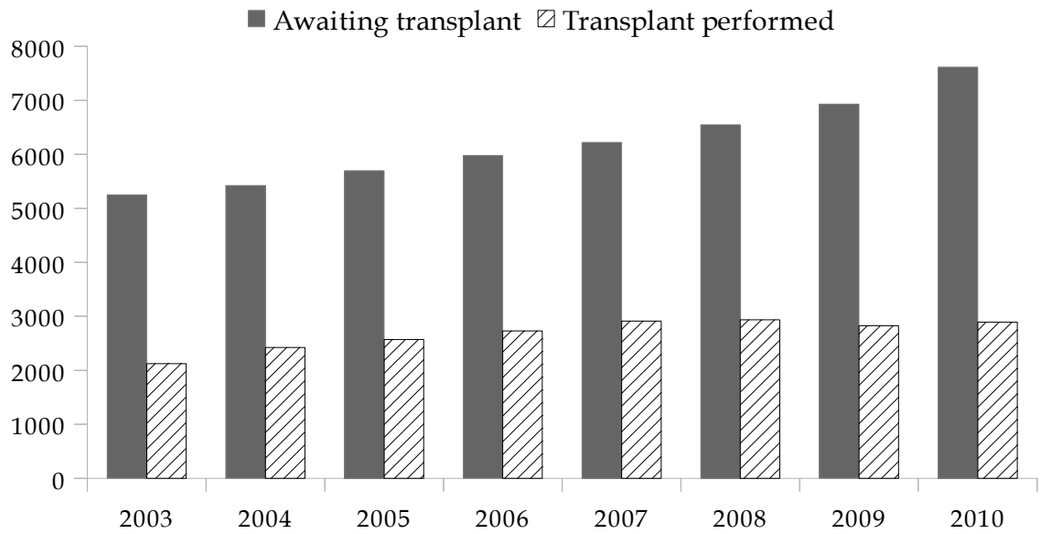


Fig. 1. Evolution of the number of patients on the waiting list for transplant and the number of transplantations performed between 2003 and 2010 in France (from Agence de la Biomédecine Rapport annuel 2011).

Although campaigns for organ donation increase the number of donors, donated organs are most often in marginal conditions because the donors often present co-morbidity factors (diabetes, obesity, cardiovascular disease, hypertension, poor renal function). In the case of the kidney, the percentage of successful grafts tends to decrease. These factors weaken the organ and render it more susceptible to develop lesions following ischemia reperfusion, the unavoidable syndrome encountered during organ preservation. As the extend of these lesions is correlated to graft outcome, it is necessary to improve the entire sequence of ischemia-reperfusion and in particular to find ways to improve storage conditions and decrease the rate of delayed graft function after kidney transplantation.

In kidney transplantation, delayed graft function is mainly due to the intensity of ischemia reperfusion lesions associated with the hypothermic and hypoxic conditions during preservation. These lesions are exacerbated at the moment of transplantation with reoxygenation that occurs during the reperfusion. In this light, tissue damages identified by the detection of endogenous metabolic changes in plasma and urine would permit a better estimation of graft quality and improved management of the graft, to the direct benefit of the patient. High Resolution Magnetic Resonance Spectroscopy (HR MRS) in these fluids could permit the identification of biomarkers indicative of specific kidney lesions occurring during an ischemia-reperfusion sequence.

The purpose of the chapter is to make a mini-review about thematics involving kidney transplantation from animals, mainly large white pig, to humans in order to highlight the potential of MRS, high resolution or imaging, and what perspectives could be envisioned to improve comprehension of kidney transplantation.

## 2. High resolution NMR

### 2.1 Kidney analysis

Before considering transplantation, it is important to review nephrologic situations and nephropathologies that have been widely analyzed by MRS to find biomarkers of pathologies associated to kidney.

In 1999, Garrod and al. established the biochemical composition of normal inner and outer renal cortex and renal papilla from rats (Garrod et al., 1999). This work realised with $^{1}H$ liquid NMR spectroscopy on tissue extracts and with $^{1}H$ hrmas (high resolution magic angle spining) NMR spectroscopy on crude tissue allowed the direct observation of metabolites into kidney and showed the metabolic composition of the 3 main areas of the kidney. They established the $_{1}H$ (hydrogen-1) and $_{13}C$ (carbon-13) chemical shift assignments of metabolites found in cortex and/or papilla. Still with $^{1}H$ hrmas NMR spectroscopy, a team realised the metabolic profiling of normal and hypertensive rat kidney (Huhn et al., 2004). They showed that better differentiation between both groups occurred in the kidney cortex. It has also been shown that NMR can be used to compare intact kidney, blood plasma and urine of 4 species of rodent. They thus showed that metabolic data acquired on laboratory animals can only be extended to wild species with the use of a lot of precaution (Griffin et al., 2000).

On those bases, and among pathologies affecting the kidney, diabetic nephropathy is one of the lethal manifestations of diabetic systemic disease (Zhao et al., 2011). Using a rat model of

diabetic nephropathy induced by streptozotocin, the authors performed a holistic metabolic analysis in order to elucidate the mechanism of this disease. For that purpose, they realised $^{1}H$ liquid NMR spectra of urine on one hand and kidney extracts on the other. Elevated level of glucose in diabetic rats affected the ketone pathway, fatty acid oxidation, tricarboxylic acid cycle and glycolosis. Those metabolic changes led to decrease of energy production, hence aggravating kidney damage. Moreover, in this study the authors assessed osmolyte metabolism.

Another cause of kidney failure is autosomal dominant polycystic kidney diseases (ADPKD). Some candidate urinary protein biomarkers such as KIM1 or NGAL have already been evaluated for this nephropathology. However, to improve the quality of diagnosis for ADPKD, complete $^{1}H$ and $^{13}C$ NMR fingerprint of urine were put in place (Gronwald et al., 2011). Elevated levels of proteins and methanol were found in patients with ADPKD receiving medication against hypertension.

NMR is well suited for the study of neprotoxicity. In 1998, the team of Nicholson reported urine metabolic profiles in 15 groups of animal treated with nephrotoxic molecules and compared spectra with control. They thus highlighted endogenous biomarkers of nephrotoxic insult (Holmes et al., 1998). Citrate, 2-oxoglutarate and hippurate were the most discriminating metabolites between treated group and control group whereas formate and dimethylamine showed almost not change. Other studies on administration of nephrotoxic compounds realised with urine and plasma of Sprague-Dawley rats focused on lanthanum, a rare earth compound used as fertilizer with a higher accumulation rate and a lower metabolic rate (Feng et al., 2002) or thioacetamide (Waters et al., 2005). In the first study, the authors showed that a 6-months long ingestion of $La^{3+}$-induced nephro- and hepato-toxicity which could be highlighting by using NMR. From a dose of 10 mg/kg of $La(NO_3)^{3+}$, all the evolving quantified metabolites involved Krebs cycle intermediates or amino acids that are potential NMR markers for $La^{3+}$-induced proximal tubular lesions. Moreover, decrease of creatinine in urine highlighted a low glomerular filtration. A second study focused on energy intermediairy (citrate, lactate, succinate) and lipid metabolisms. The work also realised on intact kidney tissue using hrmas NMR spectroscopy (Garrod et al., 2001; Wang et al., 2006) established changes in the spectral profile of renal papilla involved the marked depletion of several renal osmolytes such as glycerophosphocholine, betaine, and myo-inositol.

Finally, the effects of cyclosporine A, the basis for most immunosuppressive protocols and used for allograft recipients on kidneys were evaluated (Lenz et al., 2004; Serkova et al., 2003). This treatment is well known for its nephrotoxicity. Cyclosporine A induced a decrease of poly unsaturated fatty acid and an increase of lipid peroxidation which suggests the use of an alternative pathway for energy production when the oxidative mitochondrial pathway is inhibited. This lipid peroxidation is responsive for kidney damage especially in the cortex and medulla. Cyclosporine A also leads to osmolyte regulation (taurine, betaine and trimethylamine-N-oxide (TMAO)).

### 2.2 Kidney transplantation analysis

#### 2.2.1 Isolated Perfused Kidney (IPK) of pig

The isolated perfused pig kidney (IPK) was used to mimic the ischemia reperfusion episode occurring during transplantation. This model was destined to assess initial renal function

after different preservation condition. The schematic diagram of the isolated perfusion method is illustrated in the Figure 2. Based on an isolated perfused kidney, HR MRS injury biomarkers of an ischemia-reperfusion sequence were identified in the urine. Spectra modifications compared to histological or biochemical analysis showed changes of some metabolites excretion.

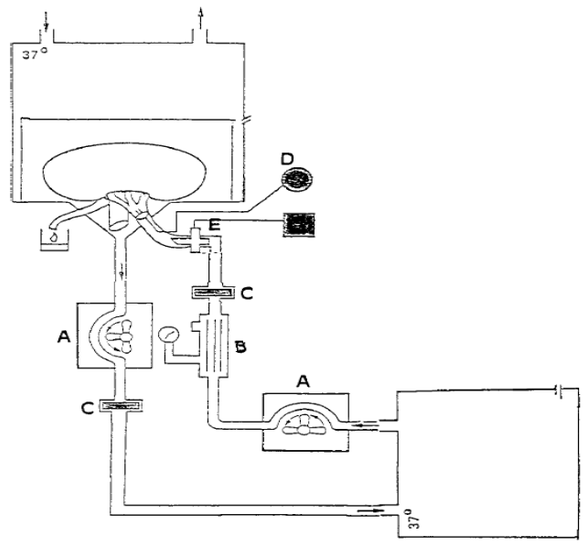


Fig. 2. Schematic diagram illustrating the isolated perfusion method in which A is the peristaltic pump, B, the oxygenator, C, the filter, D, the pressure transducer, E, the flow meter and electromagnetic flow probe (Hauet et al., 2000d).

One of the first studies using HR MRS on this model was performed to mimic the non-heart-beating donor situation. The kidney underwent a warm ischemic period before the hypothermic period of conservation and reperfusion (Hauet et al., 1997b). In this study, 3 groups of pigs were used. In the control group, kidneys were flushed with cold heparinized saline and immediately perfused. Kidney of a second group underwent a cold flush and 24 hours of cold-storage preservation (CSP) and finally reperfusion. The last group was composed of kidneys with 30 minutes of warm ischemia, 24 hours of CSP and reperfusion. The reperfusion occurred at 37°C and was performed with Kreb's solution supplemented with 22 amino acids. Among the interesting metabolites, TMAO and lactate were higher in the group with warm ischemia and related to damage of medullar cells and to tubular dysfunction respectively. Another study performed with the IPK was performed to assess the effect of cold storage conservation time with EuroCollins solution (EC) (Hauet et al., 1997a). Perfusion time was pushed to 72 hours and the degree of proximal tubule cell damage was increased with prolonged cold ischemia (Goujon et al., 1999). Urine content after a delay of 24 hours of perfusion was not significantly different from urine content at the beginning of the perfusion. After 48 hours, urine profile was significantly different. Rises of TMAO/creatinine and lactate/creatinine ratio measured with NMR were in accordance with elevated levels of beta-N-acetylglucosaminidase and lactate dehydrogenase. The same protocol was used to compare EC to University of Wisconsin solution (UW) (Hauet et al., 2000c; Hauet et al., 1999). Metabolites released into the conservation solution analyzed with NMR also confirmed classical biochemical parameters such as perfusion flow rate,

glomerular filtration rate (GFR), tubular reabsortion of sodium and lactate dehydrogenase levels (Hauet et al., 2000d).

### 2.2.2 In vivo autotransplantation pig model

The IPK model, which provided ex vivo information, was converted to a preclinical model with 3-months old large white pig. Briefly, the basic model was to remove the kidney, preserve it 24 hours and then transplant it in the same animal with a contralateral nephrectomy. We followed the animal for 3 months after transplantation and we studied biochemical parameter in order to correlate them with MRS data.

In this model, damage biomarkers appearing after an ischemia reperfusion episode were analyzed in order to confirm their pertinence with the follow up of the recovery of function. To summarize, metabolites quantified with liquid NMR were TMAO, correlated to medullar lesions, acetate correlated to cortical lesions and lactate linked to global ischemia. Amino acids such as alanine or valine could be associated with proximal tubules dysfunction. Creatinine measured in both plasma and urine allowed the determination of GFR. All these metabolites were highlighted in different studies summarized below.

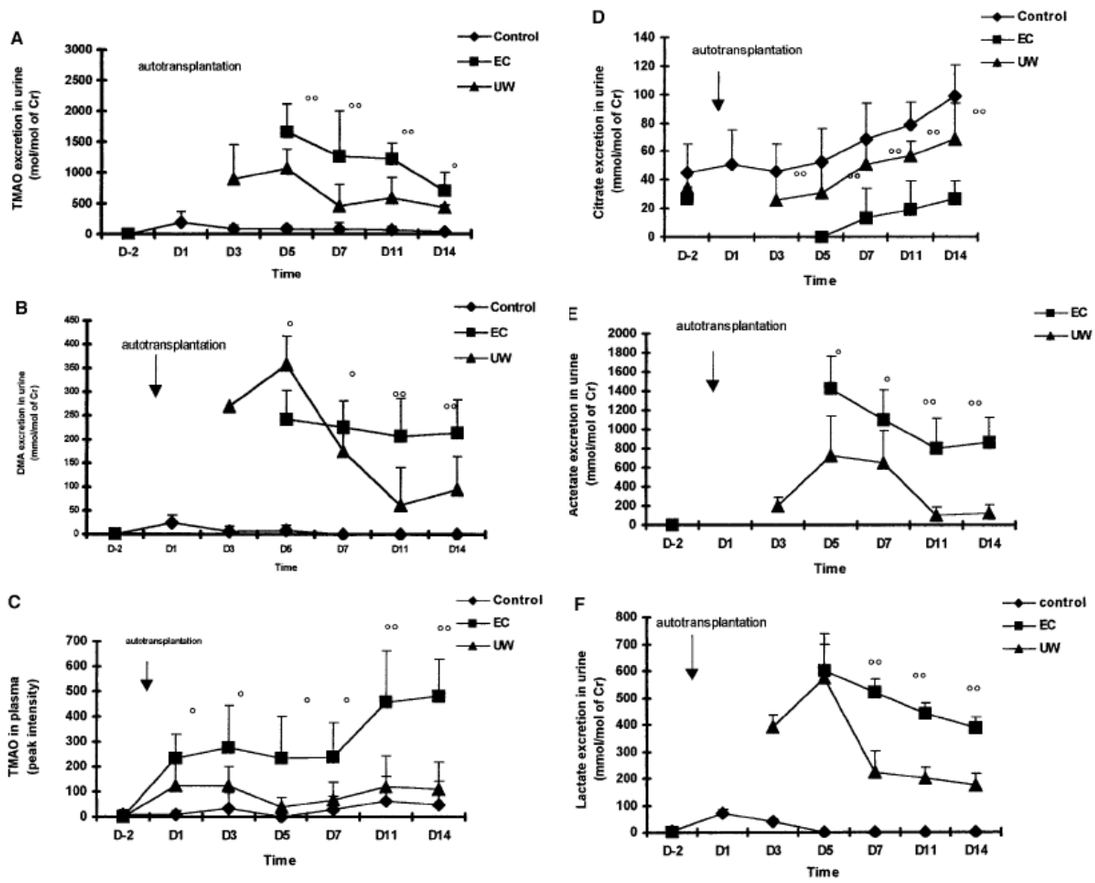


Fig. 3. Levels of trimethylamine-N-oxide (A) and dimethylamine (B) in urine and changes of trimethylamine-N-oxide in plasma (C). Levels of citrate (D), acetate (E), and lactate (F) excretion in urine after autotransplantation. °P , 0.05, °° P , 0.01 (Hauet et al., 2000b).

In 2000, comparison of EC and UW solution with CSP, already performed on IPK of pig, was performed on this preclinical model of kidney autotransplantation (Hauet et al., 2000b). The control group, undergoing uninephrectomy, presented 100% survival at day 14 whereas only 75% and 66 % of UW and EC pigs survived, respectively. Glutathione-S-transferase and creatinine clearance were significantly different between EC and UW groups with a light advantages for UW compared to control. Results obtained with MRS on urine and plasma are presented in figure 3. Delayed graft function was associated with the release of osmolytes such as TMAO in urine and in plasma. Moreover, dimethylamine, acetate, citrate and lactate level in the UW group were always closer to control group levels compared to levels in the EC group. These data outline the effect of UW preservation against renal medullar injury and impairment of oxidative metabolism. Here, strength of MRS was highlighted: in one experiment, with a 200 to 500 µL volume of sample, detection and quantitation of large number of metabolites can be made. This allows for a multivariable analysis which is an important factor for diagnostic and prognostic purpose.

Using the same experimental design, the impact of the preservation solution was demonstrated and the addition of colloids (polyethylene glycol (PEG)) into an extracellular solution showed better results (Faure et al., 2002). Indeed, in the urine spectra, TMAO/creatine ratio were significantly higher in kidney preserved with EC, UW or a solution containing PEG-50 than in kidney preserved with a solution containing PEG-30. This demonstrated that PEG reduced damages to the renal medulla during conservation, in a dose-dependent manner. Citrate was not detected during the first postoperative week in urine from kidneys preserved in EC. Its excretion was detected significantly earlier in urine from the ICPEG30 group than from the UW and ICPEG50 groups. This suggested that PEG associated to an intracellular solution improved oxidative metabolism and reduced renal medulla injury, also in a concentration-dependent manner. Furthermore, the addition of antioxidative (Baumert et al., 1999) or anticoagulant (Favreau et al., 2010) molecules, trimetazidine and melagatran respectively, into the preservation solution increased the performance of the hypothermic conservation. The effect of trimetazidine during conservation with EC or UW showed interesting results. The early excretion of acetate in the trimetazidine supplemented group demonstrated an efficient function recovery of the citric acid cycle compared with standard solutions (Hauet et al., 2000a). Combination of PEG and trimetazidine in conservation solution was the next step of long term time study which included 18 groups also exploring cold ischemia time (Doucet et al., 2004). Biological parameters indicated that reduced delayed graft was found when combining PEG and trimetazidine during CSP. Histological staining for CD4+ positive cells indicated that PEG reduced CD4+ positive cells infiltration and trimetazidine was efficient in reducing the inflammatory reaction. The NMR analysis was again in accordance with these results. Acetate excretion between day 1 and day 14 was improved in all experimental group preserved with trimetazidine while urinary TMAO was reduced in all trimetazidine group and we noticed presence of TMAO in plasma in all group except from control. Combination of trimetazidine with PEG seemed to be the best option for kidney conservation and MRS brought supplementary information to classical biochemical parameters such as GFR or sodium excretion. In addition, this study underlined that MRS permits the performance of a multivariate analysis in a single experiment.

Use of conditioning drugs before conservation was also investigated. Donor pretreatment with N-acetylcysteine (NAC) has been shown to ameliorate acute renal failure. Extractions

with perchloric acid of kidney tissue samples 24 hours after transplantation were performed and hydrosoluble metabolites were analyzed with $^{1}H$ liquid NMR spectroscopy. In parallel, blood samples were analyzed. TMAO again gave the best results. Its decreased levels in the blood of NAC group compared to control group were associated with a decrease of allantoin in the blood and kidney tissue. This latter metabolite results from the free radical action of urate and its presence indicates free radical activities. Pretreatment with NAC reduced radical excretion, hence reduced free radical activity and thus tissue damages (Fuller et al., 2004).

### 2.2.3 Application to Human

Use of HR MRS in Human to follow transplanted kidney recovery of function highlighted several issues. The main problem came from the drug treatment received by patients after transplantation. We observed overlapping between the signals of the metabolites of interest and of the drugs. In these conditions it is often difficult to extract information about the kidney function. Moreover a high variability, in addition of the individual variability, is often observed. This variability, mainly due to diet, may cause urine profile modifications which can hide variations of the metabolite of interest in relation with the kidney injuries. One solution could be to introduce a 2-days controlled diet before each sampling to minimise those environmental variation. However, an older study performed on 33 patients who underwent primary renal allograft transplantation, using the same immunosuppressive regiment and of which 57.6% achieve immediate graft function, showed that patients with good graft function and normal patients had urinary TMAO/creatinine level inferior to 200 µmol/mM while patients with graft dysfunction had urinary TMAO/creatinine level of 410 ± 102 µmol/mM (Foxall et al., 1993).

## 2.3 Perspectives

### 2.3.1 Perfusate analysis

Hypothermic machine perfusion (MP) is increasingly being preferred to CSP. Indeed, MP has been showed to offer protection to the organ since the 80's (Alijani et al., 1985; Kwiatkowski et al., 2007; Wight et al., 2003) with benefits such as reduction of delayed graft function and increased survival. However, the mechanisms involved in these improvements are still unclear and need to be elucidated. Biomarkers such as lactate dehydrogenase, total glutathione-S-transferase or N-acetyl-β-D-glucosaminidase measured at the end of the perfusion in the machine perfusates are used by clinicians. Even if those markers are independently efficient to predict graft outcome, they are not efficient enough to be taken into account in the decision to transplant or discard a donation after brain death or controlled donation after cardiac death (Moers et al., 2010; Siew et al., 2011). Again the potential of multivariate analysis offered by NMR could be used to predict graft outcome. In a recent study in a pig model of autotransplantation of livers, the effect of warm ischemia on metabolites contents into perfusats was measured (Liu et al., 2009).

Currently, our team is attempting to answer the question: can $^{1}H$ HR NMR metabonomic analysis during machine perfusion be used to predict graft outcome? We thus work on perfusates from different protocols of kidney transplantation using MP. We evaluate the effect of warm ischemia, duration of ischemia, types of solution and temperatures of preservation and finally effect of adjuvant in the perfusion solution. We showed, in a small

cohort of pig with two distinct graft outcomes, that a correlation between creatininemia at day 7 after transplantation and choline concentration after 24 hours of perfusion existed. Other metabolites like TMAO, lactate or glutathione showed the same correlation (Figure 4.). Metabonomic NMR analysis of machine perfusates could thus be use to predict graft outcome. A larger study is required to validate this finding, especially since this technique is easily transposable to the clinic.

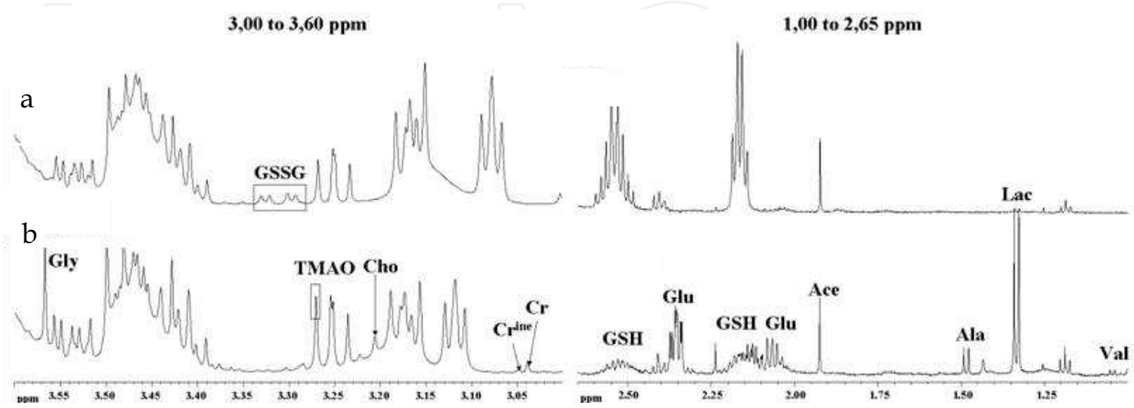


Fig. 4. $^{1}$H HR NMR spectra of kidney perfusion solution (a) at the beginning of the perfusion and (b) after 24 hours of perfusion. (Ace: acetate, Ala: alanine, Cho: choline, Cr: creatine, Cr$^{ine}$: creatinine, Glu: glutamate, GSH: reduced glutathione, GSSG: oxidized glutathione, TMAO: trimethylamine-N-oxide, Val: valine).

### 2.3.2 $^{1}$H hrmas NMR on kidney tissue or cells

In an hrmas probe, adapted for classical spectrometer, samples are spun with high speed (2000 – 5000 Hz) at a 54.7° angle. This rotation leads to decreased heterogeneity of samples and allows direct sample analysis. $^{1}$H hrmas NMR is thus particularly adapted for tissue or cells without sample pre-treatment (Desmoulin et al., 2008). Addition of deuterated water into phosphate buffer solution is the only required modification of the samples.

In regards to the in vitro analysis of ischemia reperfusion injuries, our team developed an endothelial cell model mimicking transplantation through a hypoxia-reoxygenation episode in which conditions of hypoxia and/or reoxygenation can be modulated by changing temperature of hypoxia, atmosphere or conservation solutions. $^{1}$H hrmas NMR is a precious tool for this application but needs improvement for cell analysis. Indeed, the main problem resides on cell quantity, especially after hypoxia when cell number is inferior to 1 million as it is the case in our experiments. Use of the rotor allows for limitation of volume inside the tube and is one of the solutions we are examining. On the other hand, after 30 minutes spinning at 2500 Hz at 4°C, when cells were replaced into culture medium, 40 to 60 % of cells were still alive and restarted growing normally after 24 hours. Thus, we could imagine the use of $^{1}$H hrmas NMR to study all hypoxia-reoxygenation sequence on the same cells. In parallel, supernatant can also been analyzed in order to follow the cell metabolism.

Concerning tissue analysis, care has to be taken during tissue collection. Indeed, as already mentioned, because of its histological heterogeneity the kidney has different metabolites composition depending on the area (Garrod et al., 1999). For autotransplantation models as in clinical studies, a defined protocol for biopsy must be respected by all involved parties.

## 3. Magnetic resonance in vivo

### 3.1 MRI

Magnetic resonance imaging (MRI) is a non invasive technique bringing essential information in the diagnostic of kidney transplant rejection since it can provide data on the anatomic and functional status of the transplanted kidney (Beckmann et al., 2000). Briefly, anatomic MRI is applied to evaluate the transplanted organ by assessing structural and anatomical changes or by measuring the graft volume. The most popular imaging sequences are the T1- and T2-weighted spin echo sequences and the T1-weighted gradient echo sequence. However, it would really be informative to add other acquisition sequences to obtain functional information about the transplanted kidney, if the magnet system is available during enough time to perform these exams. Indeed, further information can be obtained on perfusion and functionality of the graft and the integrity of the tissue.

In addition of the magnetic resonance angiography to image blood vessels, quantification of the renal blood flow can be performed with dynamic MRI (Montet et al., 2003). Using a contrast agent, an association of a lower MRI perfusion with chronic allograft nephropathy severity was found (Pereira et al., 2011). Perfusion was determined in chosen area of the kidney, by measuring enhanced signal in the region of interest during the injection of the contrast agent. These results led to an homogeneous estimation of the perfusion, and the ability to evaluate the entire kidney with perfusion MRI may have benefits in establishing which areas of the transplant have a reduction in perfusion. The limitation in obtaining an entire kidney image is the necessity to use a cardiac gating to avoid pulsatility in the arterial curve. Another problem is the potential nephrotoxicity of the gadolinium which is generally used as contrast agent in a chelated form. Using low dose three dimensional magnetic resonance renography, the mean transit time of the tracer for the different compartment of the kidney (vascular, tubular and collecting system) was measured different in normal kidneys, transplanted kidneys with acute tubular necrosis and transplanted kidney with acute rejection (Yamamoto et al., 2011) indicating that a multicompartimental tracer kinetic renal model may help to differentiate acute rejection from acute tubular necrosis in transplanted kidney.

Ultra-small superparamagnetic particles of iron oxides (USPIO) could be used as contrast agent in clinical medicine for in vivo MRI due to their properties to locally perturb magnetic field and novel particles are in development (Mills et al., 2011). Their toxicity is not well known and liquiq $^{1}H$ NMR analysis of plasma and urine demonstrated metabolic changes (Feng et al., 2010). Energenetic and lipidic pathways such as glucose and amino acid metabolisms were affected by USPIO administration. Whereas no change has been found in histopathological analysis of tissue between two types of USPIO administration, coated with dextran or uncoated, coated-USPIO administration led to acetate, unsaturated fatty acids and some amino acids elevation in the $^{1}H$ hrmas kidney profile. It also led to decrease of triacylglycerol, myo-niositol or taurine, for instance (Feng et al., 2011). Hence, this sort of tracer has to be used with precaution.

A noninvasive magnetic resonance arterial spin labeling (ASL) technique has been performed to evaluate kidney perfusion without contrast agents (Artz et al., 2011). ASL uses the blood as an endogenous contrast agent allowing perfusion measurements without gadolinium injection. Using a flow-sensitive alternating inversion recovery (FAIR) sequence, inflowing blood is selectively labeled to have an opposite magnetization compared to the

destination tissue. The difference between a labeled image and a non labeled image can be used to calculate tissue perfusion. The first results obtained indicated that medullar perfusion was systematically lower in transplanted versus native kidneys irrespectively of estimated glomerular filtration rate (eGFR). Cortical perfusion was lower for transplanted kidneys when eGFR was > 60 ml/min per 1.73 $m^2$ and correlated with eGFR in both native and transplanted kidneys. These results were obtained with a one model compartment requiring rapid water exchange assumption between the intravascular and extravascular space. A two compartments model would allow more accurate perfusion quantification, but would require measurements which are not possible in a clinically feasible scan time.

Diffusion weighted (DW) MRI, which was established for the tissue characterization and lesion detection (Thoeny &De Keyzer, 2011), is particularly interesting for kidney function exploration because of its high blood flow and water transport functions. Simple acquisition is performed with a bipolar gradient (diffusion gradient) dedicated to the assessment of fluid movements. When molecules of water moves, the absence of rephasement of the nuclear spin appears as a loss of signal intensity on the image. If diffusion is restricted, molecules of water reduce their movement and may be refocused by the second gradient impulse (Palmucci et al., 2011). Different intensities of diffusion gradient can be used to determine the Apparent Diffusion Coefficient (ADC). The DW MRI techniques yields a total ADC in each voxel explored that provides information on diffusion properties of water, including contribution from micro-circulation (Le Bihan et al., 1988). Thus image processing allows to separate the micro-circulation information, quantified with the perfusion fraction ($F_P$) which reflects micro-circulation of blood and movement of fluids in predefined structure such as tubules and glomeruli, and the primarily pure diffusion with the perfusion-free diffusion ($ADC_D$) (Eisenberger et al., 2010). The potential of ADC has been studied in kidney transplantation to determined modification in case of graft rejection or acute tubular necrosis. In patients with stable allograft function (posttransplant time between 8 and 10 months), total ADC and $ADC_D$ were found identical in the cortex and medulla of the transplanted kidney while values were higher in cortex than in medulla of healthy volunteers. Cortical total ADC and $ADC_D$ were higher in native kidneys than in transplanted kidneys (Thoeny et al., 2006), and a difference in $ADC_D$ was observed between patients with normal clearance (>60 mL/min) and low clearance (>30 mL/min) where $ADC_D$ were the lowest (Palmucci et al., 2011). In allografts with stable function early after transplantation (posttransplantation time of 10 days) $ADC_D$ and $F_P$ were also found identical in cortex and medulla, but the $F_P$ was strongly reduced in the cortex and medulla of renal transplants with allograft rejection and acute tubular necrosis. $F_P$ correlated with eGFR, while no correlation were found between eGFR and $ADC_D$ or total ADC (Eisenberger et al., 2010). These results show that DW MRI is a promising non-invasive method for detection or monitoring of functional derangements early after kidney transplantation.

The blood oxygen level dependent (BOLD) MRI is a functional MRI technique allowing assessment of the tissue oxygenation. When the kidney is the explored organ, it allows the differentiation of specific anatomical regions (I.E cortex, inner and outer medulla). BOLD MRI is based on susceptibility differences between oxyhemoglobin and deoxyhemoglobin which induce image contrast differences, generally obtained with a gradient echoes sequence. Oxyhemoglogin is diamagnetic, and has no effect on the NMR signal of the surrounding water, by contrast deoxyhemoglobin is paramagnetic, increasing its magnetic properties and modifying the signal relaxation of the surrounding water molecules. Higher concentrations of deoxyhemoglobin induces shorter apparent T2 relaxation time, which is in

relation with the oxygen consumption in the tissue. In a murine model, BOLD MRI of a clamped kidney showed the decreased oxygenation of all regions. 24 hours after reperfusion, a lower reoxygenation of the outer medulla than control was found, and a higher reoxygenation of the cortex and an identical reoxygenation of the inner medulla were determined (Oostendorp et al., 2011). These results highlighted the potential of BOLD MRI for the detection of change in kidney tissue oxygenation. Non invasive exploration of human kidneys showed that oxygenation was lower in medulla than in the cortex, confirming a physiological medullar hypoxia (Malvezzi et al., 2009; Thoeny et al., 2006). A few days post transplantation, this technique showed an increase of T2 relaxation times in the cortex and medulla of the transplanted kidney, compared to the donated kidney of living donor before collection, demonstrating increased oxygenation (Malvezzi et al., 2009). A few months after transplantation, an increased oxygen content in the medulla was observed despite an unchanged perfusion fraction (Thoeny et al., 2006). Comparing allografts with normal function or acute tubular necrosis, a decreased blood flow and an increased oxygen bioavailability in the medulla of acutely rejecting kidneys were observed, suggesting a greater decrease in oxygen use (Sadowski et al., 2010). No significant modification of BOLD and DW MRI were observed in patients with acute tubular necrosis compared to normal functioning allografts. These results indicate the potential of these MRI techniques to longitudinally follow transplanted kidneys and obtain information on function.

As an anecdote, MRI was tested for the counting the kidney glomerules number and size distribution in normal rat kidneys using ferritin as labeling and compared to standard stereological evaluation (Heilmann et al., 2011). This study showed that this ex vivo analysis of entire kidney was less time consuming than stereological method.

## 3.2 $^{31}$P MRS

Magnetic resonance spectroscopy of Phosphorus-31 ($^{31}$P) can be performed in kidney during the preservation period and in vivo after transplantation. In these two cases, $^{31}$P MRS brings information about the energetic metabolism. This non invasive methods allows the observation of a limited number of metabolites which are mainly: adenosine triphospate (ATP) which gives three signals corresponding to the three phosphorus nuclei in the ATP molecule ($\alpha$, $\beta$ and $\gamma$ phosphorus corresponding to the position of the phosphorus nucleus in the molecule); inorganic phosphate (Pi) which has a chemical shift varying with the intracellular pH; phosphomonoesters (PME), mainly including phosphocholine and phosphoethanolamine that is highly concentrated in the renal cortex; phosphodiesters (PDE), corresponding to the resonances of glycerophosphocholine (GPC) and glycerol-phosphoethanolamine (GPE) which can be used as an indicator of the physiological integrity of the organ (Wolff &Balaban, 1988). Phosphocreatine is not seen in NMR spectrum of kidney, and when it appears in vivo, it comes from the surrounding tissues.

Ex vivo or in vivo, $^{31}$P MRS does not allow an absolute quantification thus only signal ratios can be used. These ratios were studied in the preservation period and after the transplantation of the kidney, and compared with the kidney graft function. During the preservation period, $^{31}$P MRS was performed ex vivo with the kidney in the preservation solution and in the apparatus of conservation. The ATP disappeared quickly after the collection and perfusion with a cold preservation solution, in spite of the fact that the kidney was immediately cooled to 4°C and maintained to this temperature. The most significant

indicator of graft quality was the ratio of PME to Pi (PME/Pi), determined in human cadaveric kidneys prior to planned transplantation, which has been correlated with tubular necrosis and delayed graft function (Bretan et al., 1989; Hene et al., 1994). A high PME/Pi ratio was associated with the best renal function after transplantation; a decrease of this ratio (decrease of PME and/or increase of Pi) correlated with prolonged acute tubular necrosis. In regards to the determination of this ratio in the preserved kidney, the difficulty was the presence of a phosphate buffer in the preservation solution, this Pi signal overlapping the intracellular inorganic phosphate. Determination of this ratio can be obtained using Chemical Shift Imaging (CSI). This technique uses spatial resolution to obtain a 2D-CSI spectra, where each spectrum comes from a voxel. The interest is to minimize the Pi signal of the preservation solution in the voxel's content. In these conditions, a better correlation was obtained between PME/Pi ratio and serum creatinine 14 days after transplantation (Niekisch et al., 2004). A decrease of the PME/Pi ratio was shown as a monoexponential time dependent function in pig kidneys (von Elverfeldt et al., 2007). This decrease shows the influence of preservation time on transplantation outcome, and the authors determined a different decay constant with the preservation solution (comparison between two solutions) suggesting a predictive indicator of the preservation quality with time. This PME/Pi ratio indicator can be obtained with the kidney staying in its preservation container, at preservation temperature. No injection or biopsy is necessary, allowing easy integration of this exam in the period between collection and transplantation; the only condition being a non-magnetic container to be introduced in the magnet of a magnetic resonance apparatus (whole body scanner MR for this exam).

After transplantation it is possible to perform an in vivo $^{31}$P MRS of the kidney, which remains fully non invasive as it does not require injection nor anesthesia. More information can be obtained from the $^{31}$P spectrum of kidney since in addition to the PME and PDE signals, ATP signals can be observed and intracellular pH can be determined with the chemical shift of the Pi. When spectrum is obtained from the whole kidney, β-ATP to Pi ratio measured 2 or 3 years after transplantation has been shown to be an excellent indicator of long term survival of the transplanted kidney (3 years when ratio > 1.2 and 5 years when ration > 2.5) (Seto et al., 2001). When MRS was performed in the 4 weeks after transplantation, the PME to PDE ratio seemed to be an indicator of allograft function (Klemm et al., 1998), showing a good correlation with serum creatinine levels. High PME/PDE ratio reflects the cell membrane regeneration process occurring during the first weeks after ischemia; PME level is considered as a reflection of the membrane phospholipid metabolism with the degree of cell growth and regeneration, while in contrast PDE level correspond to intermediate metabolites formed during cell membrane degradation. A decreased PME/PDE ratio was found in patients with delayed graft function due to acute tubular necrosis, but was not observed in patient with allograft rejection. A better localization of the voxel for $^{31}$P MRS could be obtained with CSI technique (Vyhnanovska et al., 2011), allowing the discard of surrounding tissue from the kidney signal in the spectra and the reduction in result dispersion, even if resolution did not permit to distinguish cortex from medulla in the spectra. In these conditions it is possible to distinguish different types of kidney failure after transplantation: (i) acute tubular necrosis, characterized by a decrease of PDE/β-ATP, PDE/Pi and PME/Pi compared to controls; (ii) acute rejection episodes (ARE), characterized by an increase of PME/β-ATP and PME/Pi ratio compare to controls. This method permitted the demonstration that late kidney graft dysfunction patients exhibited a higher Pi/β-ATP and lower PDE/Pi and PME/Pi ratio compared with ARE patients.

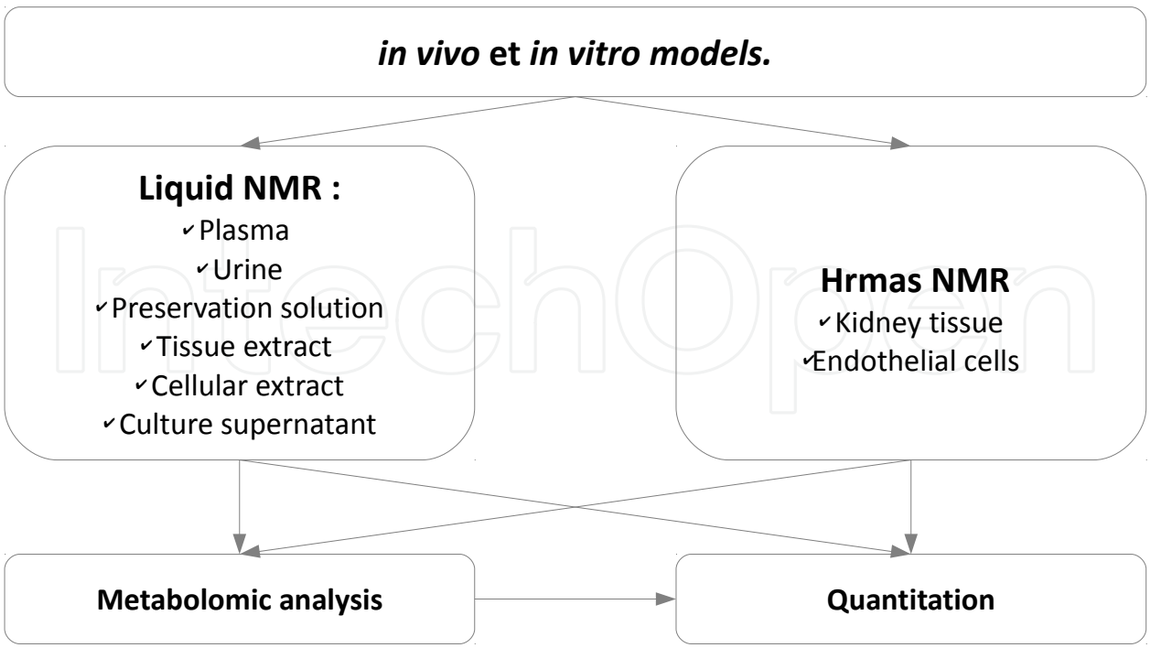


Fig. 5. Summary of samples for liquid or hrmas MRS analysis to study ischemia-reperfusion phenomena occurring during kidney transplantation.

## 4. Conclusion

From liquid NMR analysis of urine in IPK or perfusate in an autotransplantation model of large white pig, to hrmas analysis of cell culture or biopsy from Human (abstracted in the figure 5.) through imaging, MRS, combined with metabolomic analysis is a powerful tool for the study of transplantation. Despite all the difficulties in regards to kidney heterogeneity or low detection limit for cell analysis, MRS is still an interesting implement deserving of amelioration. Advances concerning imaging and contrast agents as well as transposition of hrmas to imaging are also the key for better diagnosis, prognosis and transplantation comprehension.

## 5. Acknowledgment

We thank all people working in the INSERM U927 unit and allow us to access in vitro or in vivo samples: William Hebrard, Sébastien Giraud, Frédéric Favreau, Nicolas Chatauret and Raphael Thuillier, for his grammatical help especially. Thank to Nadège Boildieu, technician performed almost all the NMR acquisition in the team.

## 6. Abbreviations

| | |
|---|---|
| $^{1}H$ | Hydrogen-1 |
| $^{13}C$ | Carbon-13 |
| $^{31}P$ | Phosphorus-31 |
| ADC | Apparent Diffusion Coefficient |
| $ADC_D$ | Perfusion-free diffusion |
| ADPKD | Autosomal dominant polycystic kidney diseases |

| | |
|---|---|
| ARE | Acute rejection episode |
| ASL | Arterial spin labeling |
| ATP | Adenosine triphospate |
| BOLD | Blood oxygen level dependent |
| CSI | Chemical Shift Imaging |
| CSP | Cold-storage preservation |
| DW | Diffusion weighted |
| EC | EuroCollins solution |
| eGFR | Estimated glomerular filtration rate |
| $F_p$ | Perfusion fraction |
| GFR | Glomerular filtration rate |
| GPC | Glycerophosphocholine |
| GPE | Glycerophosphoethanolamine |
| hrmas | High resolution magic angle spinning |
| IPK | Isolate perfused kidney |
| MP | Machine perfusion |
| MRI | Magnetic resonance imaging |
| MRS | Magnetic Resonance Spectroscopy |
| NAC | N-acetylcysteine |
| NMR | Nuclear Magnetic Resonance |
| PDE | Phosphodiesters |
| PEG | Polyethylene glycol |
| Pi | Inorganic phosphate |
| PME | Phosphomonoesters |
| TMAO | Trimethylamine-N-oxide |
| USPIO | Ultra-small superparamagnetic particles of iron oxides |
| UW | University of Wisconsin solution |

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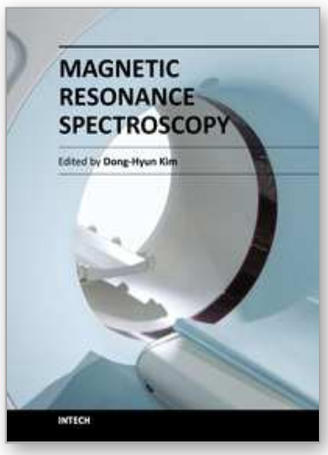


**Magnetic Resonance Spectroscopy**

Edited by Prof. Dong-Hyun Kim

ISBN 978-953-51-0065-2
Hard cover, 264 pages
**Publisher** InTech
**Published online** 02, March, 2012
**Published in print edition** March, 2012

Magnetic Resonance Spectroscopy (MRS) is a unique tool to probe the biochemistry in vivo providing metabolic information non-invasively. Applications using MRS has been found over a broad spectrum in investigating the underlying structures of compounds as well as in determining disease states. In this book, topics of MRS both relevant to the clinic and also those that are beyond the clinical arena are covered. The book consists of two sections. The first section is entitled 'MRS inside the clinic' and is focused on clinical applications of MRS while the second section is entitled 'MRS beyond the clinic' and discusses applications of MRS in other academic fields. Our hope is that through this book, readers can understand the broad applications that NMR and MRS can offer and also that there are enough references to guide the readers for further study in this important topic.



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