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## RESEARCH ARTICLE

# Detection of ATP by “in line” $^{31}\text{P}$ magnetic resonance spectroscopy during oxygenated hypothermic pulsatile perfusion of pigs’ kidneys

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## Abstract

**Object** To demonstrate that adenosine triphosphate (ATP), which provides a valuable biomarker for kidney viability in the context of donation after cardiac death (DCD) transplantation, can be detected by means of  $^{31}\text{P}$  magnetic resonance spectroscopy (MRS) if kidneys are perfused with oxygenated hypothermic pulsatile perfusion ( $\text{O}_2+\text{HPP}$ ).

**Materials and methods** Porcine kidney perfusion was carried out using a home made, MR-compatible HPP-machine. Consequently, kidney perfusion could be performed continuously during magnetic resonance imaging and magnetic resonance spectroscopy recording.  $^{31}\text{P}$  MR spectroscopy consisted of 3-dimensional chemical shift imaging (CSI), which allowed for the detection of ATP

level in line.  $^{31}\text{P}$  CSI was performed at 3 tesla in 44 min with a nominal voxel size of 6.1 cc.

**Results**  $^{31}\text{P}$  CSI enabled the detection of renal ATP when  $\text{pO}_2$  was equal to 100 kPa. With  $\text{pO}_2$  of 20 kPa, only phosphomonoester, inorganic phosphate and nicotinamide adenine dinucleotide could be found. Semi-quantitative analysis showed that ATP level was 1.3 mM in normal kidney perfused with  $\text{pO}_2$  of 100 kPa.

**Conclusions** This combined technology may constitute a new advance in DCD organ diagnostics prior to transplantation, as it allows direct assessment of ATP concentration, which provides a reliable indicator for organ bioenergetics and viability. In this study, kidneys presenting no warm ischemia were tested in order to establish values in normal organs. The test could be easily integrated into the clinical environment and would not generate any additional delay into the transplantation clinical workflow.

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**Keywords** ATP · Oxygenated HPP · Kidney · Hypothermia ·  $^{31}\text{P}$  MRS

## Abbreviations

ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
CSI	Chemical shift imaging
CSS	Cold static storage
DCD	Donation after cardiac death
FFT	Fast Fourier transform
HES	Hydroxyethyl starch
HPP	Hypothermic pulsatile perfusion
HTK	Histidine-tryptophane-ketoglutarate
KPS-1	Kidney perfusion solution
mM	Millimolar
MRI	Magnetic resonance imaging

MRS	Magnetic resonance spectroscopy
NAD(H)	Nicotinamide adenine dinucleotide
Pi	Inorganic phosphate
bPi	Buffer's inorganic phosphate
oPi	Organ's inorganic phosphate
PME	Phosphomonoester
pO <sub>2</sub>	Partial oxygen pressure
SAGE	Spectroscopy analysis by general electric
SE	Spin echo
T	Tesla
TE	Echo time
TR	Repetition time
WI	Warm ischemia

## Introduction

Transplantation using organs from donation after cardiac death (DCD), where death has been accompanied by possible irreversible damages due to circulatory arrest, often increase the risk of delayed or primary non-function of the graft [1, 2]. The possibility of using these organs, however, might significantly increase the number of kidneys available for transplantation. To make optimal use of this potential organ supply, the ischemic injury that occurs after a period of warm ischemia (WI) needs to be reversed. It has been demonstrated that oxygenated hypothermic pulsatile perfusion (O<sub>2</sub>+HPP) can restore energetic cellular levels in damaged kidneys due to prolonged WI [3–5].

Since Bretan et al. [6, 7] we know that only phosphomonoester (PME) can be detected by <sup>31</sup>P magnetic resonance spectroscopy (MRS) in organs preserved under cold static storage (CSS), [6–8] which corresponds to a condition without perfusion. The PME peak contains principally cell membrane substrates (phosphocholine and phosphoethanolamine), sugar phosphate and adenosine-monophosphate (AMP) [9]. This latest intervenes in the synthesis of adenosine-triphosphate (ATP). Thus, PME represents in part a precursor energy pool that can be drawn to regenerate ATP during reperfusion of the kidney. Moreover, it has been established that the ratio of PME to inorganic phosphate (Pi), PME/Pi has a predictive value of kidney function after the graft [6]. Hence, a minimum amount of PME (PME/Pi ratio of 0.3 as shown by Bretan et al. [6]) is required at the time of reperfusion to guarantee renal function after the graft.

Precursors of ATP are found in PME, NAD(H),  $\alpha$ -ATP and  $\gamma$ -ATP. Regarding ATP precursors, many authors have additionally shown that PME/Pi is difficult to interpret and cannot be extrapolated regardless the perfusate nature [10, 11].

It was also shown that oxygenated hypothermic pulsatile perfusion (O<sub>2</sub>+HPP) could “reanimate” the marginal organs before transplantation [10, 12–15]. Therefore, a direct measure of ATP concentration would be more advantageous to assess organ reanimation during perfusion.

In this paper, we present the results of ATP levels at 4 °C obtained with a home made O<sub>2</sub>+HPP MR-compatible machine [16] on porcine kidneys. In our study, only kidneys presenting no WI were used in order to establish the ATP level in recognized normal organs for transplantation. It is an experiment conducted to obtain basic information necessary for the next steps in marginal organs diagnostics.

## Materials and methods

### O<sub>2</sub>+HPP

The perfusion machine has been constructed using non-magnetic materials. The drive and feed-back control systems are pneumatically controlled. Detailed description of the system is presented elsewhere [17].

Ten kidneys from young pigs (age range: 4–5 months) were used for this study. All animal procedures were approved by the ethical committee of the institution's review board (Authorization No. 1052/3352/1). Kidneys were removed “en-bloc” after cold flushing with 2 l of histidine tryptophane ketoglutarate (HTK) solution (Custodiol<sup>®</sup> HTK, Dr. Franz Köhler, Chemie GmbH, Germany). The mean weight of the pigs was 30 kg. The mean weight of the kidneys was 75 g. With this procedure, no kidneys suffered from WI.

Kidneys were perfused during 8 h at 4 °C, duration generally admitted as necessary for proper reanimation [18]. The perfusion medium was KPS-1 (Organ Recovery Systems, Chicago, IL, USA) modified by the addition of 40 units of insulin per liter [19]. The perfusion medium had the same composition as the Belzer-MP medium. In particular, it contains 25 mM of phosphate. Organ perfusion requires 2 buffers: The first is a hydroxyethyl starch (HES) that is a large colloid molecule (molecular weight of 100–1,000 kilodaltons), which is retained in the vascular space. The second is a phosphate buffer that diffuses freely in the interstitial space [20, 21]. The association of both is necessary to increase the buffering capacity of the solution [22].

The maximum systolic pressure was maintained at 45 mmHg and the diastolic pressure at 15 mmHg. The frequency depended on the vascular resistance and was regulated based upon the vascular space.

After the 8 h of perfusion, the perfusion module was introduced into the MRI apparatus for <sup>1</sup>H imaging and <sup>31</sup>P spectroscopy acquisitions. The kidneys were continuously perfused at 4 °C during the entire MR procedure.

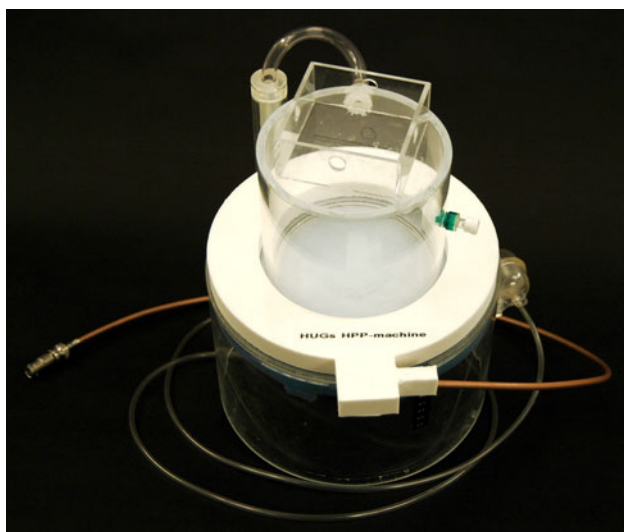
Three situations were studied: the first, perfusion with  $pO_2$  at 100 kPa (membrane oxygenator of  $0.15 \text{ m}^2$ ); the second with only surface oxygenation of the perfusate ( $pO_2 = 50 \text{ kPa}$ ); and the last at an initial  $pO_2$  of 20 kPa to mimic the CSS situation.

To determine whether ATP can be resynthesized after a period of cold ischemia, one of the kidneys perfused with  $pO_2$  of 100 kPa was further exposed for an additional period of 10 h of CSS (without perfusion) followed again by 8 h of oxygenated (100 kPa) reperfusion. Three time points after kidney harvesting could thus be obtained: at 8 h, which corresponds to cold  $O_2$ +HPP condition, at 18 h corresponding to 10 h of CSS and at 26 h corresponding again to 8 h of  $O_2$ +HPP.

### $^{31}\text{P}$ MR spectroscopy

The measurements were performed on a clinical 3-tesla system (Magnetom Tim-Trio, Siemens Medical Solutions, Erlangen, Germany) with a homemade 20 cm diameter surface coil tuned at 49.5 MHz. The coil is part of the perfusion machine (Fig. 1). It is fixed at the bottom of the “perfusor”, which constitutes the disposable piece of the perfusion device.

The coil was interfaced with a specially designed transceiver allowing both  $^1\text{H}$  imaging and  $^{31}\text{P}$  spectroscopy.  $^1\text{H}$  imaging was performed with the body coil and consisted of a T2 sequence (turbo SE, TR 5,000 ms, TE 108 ms, 3 mm slices). The field homogeneity was optimized with automatic shimming over the kidneys.  $^{31}\text{P}$  MRS consisted of 3D CSI, FOV  $250 \times 250 \times 200 \text{ mm}^3$ , matrix size  $16 \times 16 \times 8$ , nominal spatial resolution  $15.6 \times 15.6 \times 25 \text{ mm}^3$ , TR 1,000 ms, bandwidth 2,000 Hz, 1 K



**Fig. 1** Bottom view of the perfusor with the  $^{31}\text{P}$  surface coil placed underneath the perfusor (up side down)

sampling points. A Hanning-weighted  $k$ -space acquisition scheme was applied to reduce spatial contamination from adjacent voxels while improving the signal-to-noise ratio [23]. Reference transmitter  $^{31}\text{P}$  power was adjusted on non-localized  $^{31}\text{P}$  spectra (TR 10,000 ms) to get maximum buffer Pi (bPi) signal, which can be considered as homogeneously distributed over the surface coil. This reference power setting used a hard pulse of 500  $\mu\text{s}$ . It was fixed and kept constant for all the experiments. For the 3D-CSI acquisitions, a selective pulse of 3 ms was used. The pulse amplitude was scaled automatically (part of the standard sequence), and the flip angle was reduced to 40 degrees to minimize T1 saturation effect. The slice thickness was kept to the maximum (200 mm) in order to reduce the chemical-shift artefact. The pulse duration together with the phase encoding gradient duration produced an acquisition delay (TE) of 2.3 ms. Finally, a frequency offset of  $-500 \text{ Hz}$  (frequency carrier was set between the  $\gamma$ - and  $\alpha$ -ATP resonances) was used to minimize the effect of the excitation pulse bandwidth.

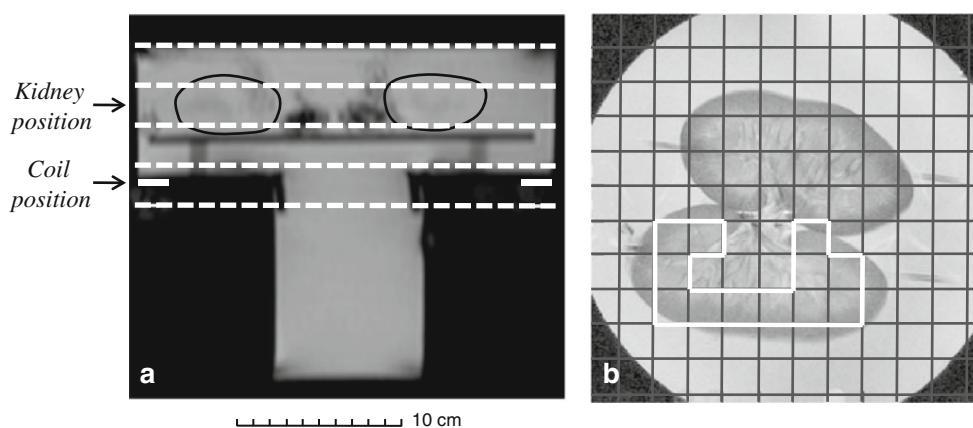
The kidneys were placed on a holder 2.5 cm above the surface coil to avoid strong RF inhomogeneities (hot spots) as well as strong  $B_0$  field inhomogeneity regions due to the perfusor shape. The kidneys were entirely covered by perfusion medium to minimize air-tissue susceptibility artefacts. Kidney position with the superimposed CSI grid are shown in Fig. 2. The same acquisition was repeated with no kidney in order to obtain the sensitivity profile of the surface coil.

Data were processed off line using the SAGE software (General Electric Medical Systems, Milwaukee, WI). The processing consisted of zero filling (2 K points), 10 Hz gaussian apodization, and FFT. First order phase correction and *sinc* spectrum deconvolution were performed to account for the acquisition delay (2.3 ms) [24]. The spectra located in the kidney were corrected for frequency shifts, and averaged. Between 8 and 12 voxels were averaged to produce a single experimental data (Fig. 2). Chemical shift is referenced to the Pi resonance set at 5.2 ppm, corresponding to buffer pH of 7.35.

Peak area (estimated by Gaussian line fitting using the Marquardt algorithm) of the kidney were normalized to bPi (25 mM) obtained from regions outside the kidneys. Coil sensitivity correction was performed to account for differences in location of kidney and buffer voxels. For each kidney, a sensitivity correction factor ( $C_{\text{sens}}$ ) was derived by dividing the average Pi peak integral of buffer voxels with the average Pi peak integral of voxels corresponding to kidney locations. In general, 2–4 buffer voxels were used. The metabolite concentration [ $^{31}\text{P}_m$ ], expressed as mmol/L (mM), was calculated as:

$$[^{31}\text{P}_m] = (S_m/S_{\text{bPi}}) \cdot [^{31}\text{P}_{\text{buffer}}] \cdot C_{\text{sens}}$$

**Fig. 2 a** Sagittal scout image showing the kidney position in the perfusor. The kidneys are placed on a holder 2.5 cm from the coil to avoid RF hot spots and magnetic field inhomogeneities. The CSI slice thickness was 2.5 cm (*dashed line*). Kidneys were surrounded by preservation fluid. **b** Coronal T2 image with the superimposed CSI grid.  $^{31}\text{P}$  spectra were obtained by voxel averaging over the kidney, excluding the hilus (*white box*)



where  $S_m$  and  $S_{b\text{Pi}}$  are the mean metabolite, respectively bPi signals (area), and  $[\text{}^{31}\text{P}_{\text{buffer}}]$  is the buffer phosphate concentration (25 mM).

The  $\beta$ -ATP area, which contains information exclusively from ATP, was used to assess ATP levels. No correction for saturation effects (T1) was attempted.

## Results

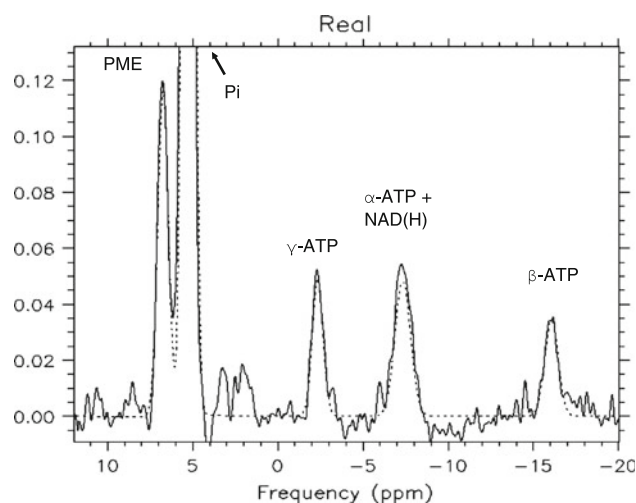
One kidney was discarded due to the presence of many cystic lesions. The remaining nine kidneys were divided into 3 groups of three, corresponding to three perfusion conditions ( $p\text{O}_2 \geq 100$  kPa,  $p\text{O}_2 \sim 50$  kPa and initial  $\text{PO}_2 \leq 20$  kPa). MRS examination was always performed 8 h after harvesting.

Presence of ATP under  $p\text{O}_2$  of 100 kPa is demonstrated in Fig. 3, which shows well-resolved resonances of  $\alpha$ -ATP (−7.5 ppm),  $\beta$ -ATP (−16 ppm) and  $\gamma$ -ATP (−2.5 ppm). Inorganic phosphate (Pi) resonates at 5.2 ppm. The Pi peak has been normalized to a value of 1 for comparison. PME peak (Membrane phospholipids, sugar phosphate and AMP) is identified at 7 ppm. The chemical shift of Pi (5.2 ppm) was used as reference. Gaussian line fitting is overlaid in *dotted line*

Figure 4 represents a  $^{31}\text{P}$  spectrum obtained with surface oxygenation ( $p\text{O}_2 \sim 50$  kPa). We observe PME and Pi peaks as well as a significant decrease of ATP level.

If we decrease the  $p\text{O}_2$  even further to  $\sim 20$  kPa corresponding to the CSS condition (Fig. 5) we observe a PME peak, Pi and also a small peak at −8 ppm, the signature of NAD/NADH products. There was no detectable ATP level.

The assessment of the metabolite concentration using the bPi area as an external reference yields an ATP ( $\beta$ -ATP) concentration of 1.3 mM with 100 kPa of  $p\text{O}_2$ . The PME level was 2.9 mM with  $p\text{O}_2$  of 100 kPa, 3.6 mM with  $p\text{O}_2$  of 50 kPa and 3.4 mM with  $p\text{O}_2$  of 20 kPa. These results are summarized in Table 1, which represents averages and standard deviation ( $n = 3$ ) of the metabolite levels.

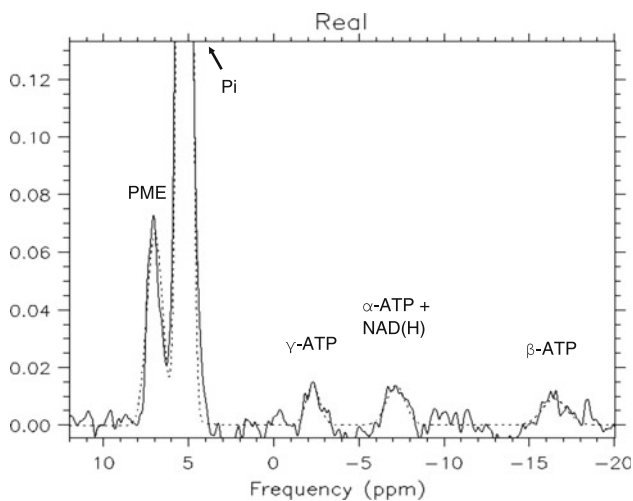


**Fig. 3**  $^{31}\text{P}$  CSI spectra of a kidney during HPP with  $\text{O}_2$  pressure of 100 kPa. The 3 ATP resonances are detected at −16 ppm ( $\beta$ -ATP), −7.3 ppm ( $\alpha$ -ATP) and −2.3 ppm ( $\gamma$ -ATP). Inorganic phosphate (Pi) resonates at 5.2 ppm. The Pi peak has been normalized to a value of 1 for comparison. PME peak (Membrane phospholipids, sugar phosphate and AMP) is identified at 7 ppm. The chemical shift of Pi (5.2 ppm) was used as reference. Gaussian line fitting is overlaid in *dotted line*

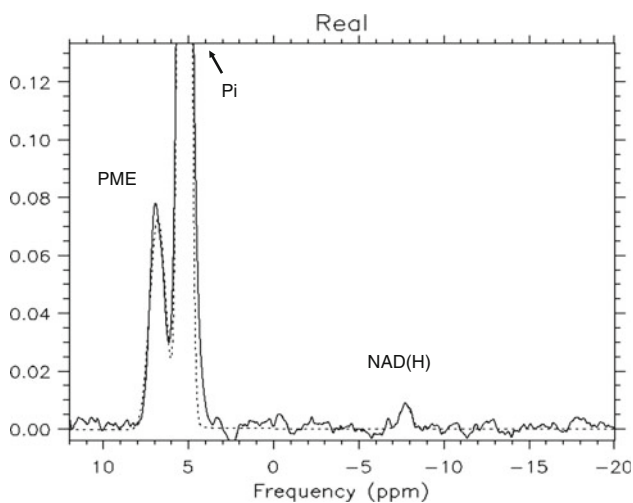
Figure 6 illustrates the effects of cold ischemia and reperfusion on ATP level after an initial period of  $\text{O}_2$ +HPP. The first  $^{31}\text{P}$  acquisition (Fig. 6a) represents semi-quantitative evaluation of the PME and ATP levels upon  $\text{O}_2$ +HPP. The second acquisition (Fig. 6b) was realized after an additional 10 h of CSS, corresponding to cold ischemic condition. ATP is not visible, only PME and NAD(H) resonances are present. Finally, the kidney was re-perfused for 8 more hours with  $\text{O}_2$ +HPP (Fig. 6c). This third acquisition was realized 26 h after surgery. In this situation, ATP was clearly detected again, and was almost back to pre-ischemic level.

## Discussion

A prerequisite for successful kidney transplantation is the viability of the donor grafts, which highly depends on the



**Fig. 4**  $^{31}\text{P}$  CSI spectra of a kidney during HPP with surface oxygenation ( $\text{pO}_2$ : 50 kPa). Only Pi (truncated, normalized to 1) and PME are visible. The three ATP resonances are visible, but with reduced levels with respect to 100 kPa  $\text{pO}_2$  (Fig. 3)



**Fig. 5**  $^{31}\text{P}$  CSI spectra of a kidney during CSS without oxygen ( $\text{pO}_2$ : 20 kPa). Only Pi (truncated, normalized to 1) and PME are visible, with a slight NAD(H) residue ( $-7.8$  ppm)

storage method. CSS together with preservation fluid (Viaspan) can safely preserve kidneys from brain dead donors (presenting no WI) up to 30 h.

With the advent of marginal organ transplantation (DCD and elderly donors), unknown factors such as prolonged ischemia may further alter kidney function. Therefore, reliable assessment of organ viability before transplantation becomes critical.

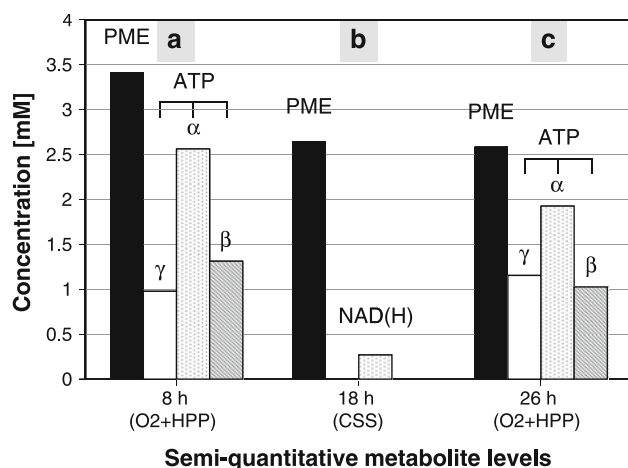
$^{31}\text{P}$  MRS was proposed decades ago as a means to non-invasively assess the viability of grafts prior to transplantation. After reperfusion, viable cells should be able to rephosphorylate AMP present in the PME to ATP. Thus, high PME may reflect in part energetic reserve for ATP resynthesis. Therefore, the ratio PME/Pi was suggested as a marker for organ viability [6, 9]. However, this ratio is difficult to assess in practice, because first, the observed organ Pi (oPi) resonance receives contribution from intra- and extra-cellular components and the difference in pH cannot necessarily separate the two entities, and second, the PME peak is made of various chemicals, which may counterbalance each other. Improved spectral resolution and the use of CSI may improve the resolution of intra- and extra-cellular Pi components [8]. Nevertheless, the assessment of intra-cellular Pi is still dependent upon the magnetic field homogeneity, which can vary drastically across the field of view as well as from one examination to another, leading to measurement inconsistency. We used a preservation fluid with a fairly high concentration of phosphate (25 mM), and with constant pH ( $\text{pH} = 7.35$ ). Measurements with a pH meter done before and after the experiment confirmed that the pH remained high (less than 0.3 pH unit variation after 24 h of perfusion). Under these conditions, it is not possible to resolve bPi from oPi peaks. A simple simulation taking into account the field homogeneity typically present in our experiments and a maximum Pi shift of 0.7 ppm (corresponding to ischemic conditions with a pH of 6.8) shows that organ Pi concentration should be at least equal to the buffer phosphate concentration in order to resolve both resonances. We are far from this situation, and one way to improve intracellular Pi assessment would consist in using phosphate-free buffer [8]. Unfortunately, such solutions are not well-suited for perfused organ preservation used in transplantation.

The use of the PME peak to indirectly assess AMP is also limited due to overlapping resonances. One way to improve the spectral resolution of the PME peak is to use

**Table 1** PME and ATP levels in mM of perfused kidneys with respect to  $\text{O}_2$  pressure

Oxygen pressure	PME	oPi	$\gamma$ -ATP	$\alpha$ -ATP+NAD(H)	$\beta$ -ATP	Time of perfusion
$\text{pO}_2 < 20$ kPa	3.36 (0.35)	21.89 (2.35)	–	0.21 (0.09)	–	8 h
$\text{pO}_2 = 50$ kPa	3.62 (0.60)	22.12 (1.98)	0.42 (0.25)	0.59 (0.25)	0.57 (0.28)	8 h
$\text{pO}_2 = 100$ kPa	2.89 (0.77)	21.99 (2.34)	1.17 (0.28)	2.09 (0.33)	1.32 (0.11)	8 h

The values are given as mean mM  $\pm$  standard deviation (in brackets). The metabolite levels were normalized to the buffer Pi area estimated at 25 mM, after correction for coil sensitivity profile. No correction for signal saturation was performed



**Fig. 6** Semi-quantitative  $^{31}\text{P}$  assessment of kidney metabolites obtained at 8, 18 and 26 h post harvesting, corresponding to 8 h of  $\text{O}_2+\text{HPP}$  (a), followed by 10 h of CSS (b), followed again by 8 h of  $\text{O}_2+\text{HPP}$  (c), respectively. Only PME and NAD(H) were observed upon CSS (condition without perfusion). ATP resynthesis was evidenced by  $^{31}\text{P}$  CSI when the kidney was reperfused with  $\text{O}_2+\text{HPP}$

$^1\text{H}$  decoupling [25]. This requires additional hardware, which is not available on our current setup.

$^{31}\text{P}$  ATP synthesis has been demonstrated in the past, under arterial blood perfusion applied immediately after kidney harvesting [26], but this model has no clinical application. In our study, we show that ATP can be detected by  $^{31}\text{P}$  CSI under fully clinical kidney preservation conditions, if appropriate  $\text{O}_2$  tension is applied to the preservation medium. This approach provides direct evidence of cell bioenergetics and hence organ viability. In addition, the measure of ATP by  $^{31}\text{P}$  MRS is much easier than the measurement of intracellular Pi and AMP (no overlapping resonances) and should improve the assessment of organ viability before transplantation.

The attempt to assess ATP concentration yields a value of 1.3 mM, which is lower than values around 2 mM reported in vivo [27, 28]. These low values may be related to hypothermia where the metabolic activity is reduced to 10–15 % of the normal level. It is also possible that ATP is not fully regenerated by  $\text{O}_2+\text{HPP}$  because of cell ischemia and mitochondrial damage that could have occurred before harvesting [12]. On the other hand, the PME value (2.9 mM) is more elevated than the concentrations found in vivo by the same groups ( $2.6 \pm 0.31$  mM). A possible explanation is that a portion of ATP is already dephosphorylated to AMP which gives rise to PME. It is of interest to note that PME is even more elevated with reduced oxygen pressure. ATP concentration was based on  $\beta$ -ATP area because it is the only resonance that represents exclusively ATP:  $\gamma$ - and  $\alpha$ -ATP peaks may have a contribution from ADP, and  $\alpha$ -ATP shares the same resonance than NAD(H). In our current experimental conditions, we

have observed a smaller  $\gamma$ -ATP resonance than  $\beta$ -ATP (Table 1), which is unlikely to occur. One possible explanation may be due to relative T1 difference between ATP moieties, as shown in muscle [29]. Another possible, indeed more likely, explanation comes from incomplete baseline correction, which underestimates the  $\gamma$ -ATP area. The error on  $\gamma$ -ATP can be avoided by the use of non-selective hard pulse to reduce the acquisition delay.

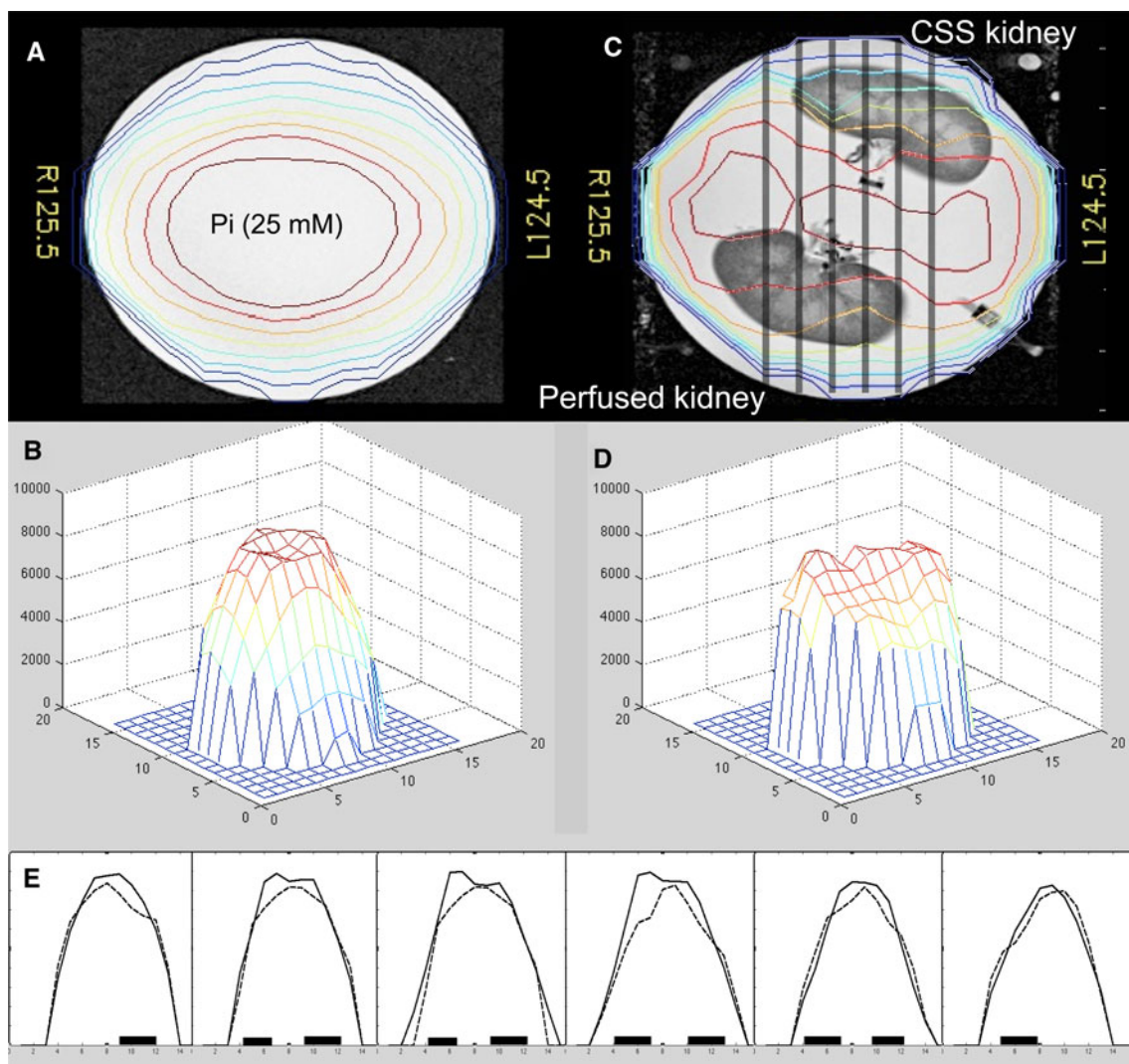
Although preliminary, these findings suggest that the bPi peak integral may provide a simple means to assess the metabolite concentration of kidneys before transplantation. The goal in this study was to normalize the data in order to compare the findings from different experimental conditions rather than provide absolute concentrations. In particular, we did not take into account differences in longitudinal relaxation time (T1) of the metabolites with respect to bPi. T1 effect is difficult to correct as it depends on the chemical compounds, temperature, organ status (ischemia) and sequence parameters (TR, flip angle). In addition, T1 measurement would need exceeding measurement time, during which the stability of the metabolite levels could not be guaranteed (although slow, metabolite levels may decrease under  $\text{O}_2+\text{HPP}$ ). However, although not absolutely quantitative, our experimental conditions (constant T1 and small flip angle) are likely to provide consistent data allowing the assessment of ATP under different kidney conditions.

This semi-quantitative approach further demonstrates the ability for ATP to resynthesize upon oxygenated perfusion. Figure 6 reports a follow-up experiment conducted on one of the kidney perfused immediately after harvesting and followed for 26 h. After an initial period of  $\text{O}_2+\text{HPP}$ , where ATP could be detected, a prolonged cold ischemic period of 10 h was carried out showing a full depletion of ATP. We observed an almost complete recovery of ATP ( $\sim 80$  %) after another 8 h of  $\text{O}_2+\text{HPP}$ . This finding has important clinical implication in the sense that cell metabolism seems to be preserved during cold ischemia, at least if the organ is subjected to prior  $\text{O}_2+\text{HPP}$  storage. Here, we observe a resynthesis of ATP in the absence of WI. However, it has been reported that better ATP recovery and graft function might be achieved with machine perfusion even when the organ is subjected to various periods of WI [30].

In the present study, bPi was measured from voxels containing no kidney tissue. The advantage of using bPi is that phosphorus concentration is known exactly (25 mM). However, correction for the sensitivity profile of the coil must be introduced to account for differences in voxel location between the kidney and the reference. The sensitivity profile was obtained from the same CSI acquisition without kidneys. The bPi area from each voxel contained in the slice of interest was then mapped (Fig. 7a, b). Because

we can assume that the coil load does not change from experiment to experiment (given the same amount of buffer), a single calibration map was obtained and used for all measurements. From these measures, organ Pi (oPi) could be calculated. On average, a concentration of 22 mM was found (Table 1), which corresponds to 88 % of the bPi concentration. This value corresponds to the Pi occupancy within the kidney. It depends on the phosphate solution used as perfusate and its capacity to diffuse in the kidney [11]. In our case, oPi is on average 88 % of the bPi, a value close to the water content of the kidney, which is known to be between 83 and 89 % depending on cortical or medullar tissue [31, 32]. Furthermore, the comparison between the Pi profile within the kidney (Fig. 7c, d) and the profile of

the empty perfusor (Fig. 7a, b) shows that Pi content is only slightly reduced. Integral difference of Pi distribution (Fig. 7e) shows that oPi is between 81 and 89 % of the bPi. This consistent result confirms that the phosphate solution used in the present study diffuses freely through the capillary bed into the interstitial space and equilibrium is attained between intra- and extra-cellular space [20, 21]. As a result, oPi as measured in the present study reflects primary buffer Pi and the intracellular phosphate generated as an end product of energy metabolism contributes only partially to the MR peak. In the future, we will further investigate whether oPi could be used as an internal reference for quantification. The later approach, if valid, would avoid the need to correct for coil sensitivity profile.



**Fig. 7**  $^{31}\text{P}$  sensitivity profile of the coil. **a, b** Represent the isocontour and mesh plots of the empty perfusor, **c, d** represent the profile with 2 kidneys in the perfusor (one under simple cold storage (CSS), and the other under oxygenated HPP). From the individual profiles (**e**) corresponding to the cross section depicted in **c**, oPi concentration could be

computed. 89 % of the bPi was present in the perfused kidney, and 81 % of the bPi was in the CSS kidney (*solid line*: empty; *dashed line*: with kidneys). The *black boxes* in **e** indicate the kidneys' location

The present findings constitute the normal basis for standard kidney harvesting, presenting no WI after 8 h of  $O_2$ +HPP. Because perfusion is not halted during the MRI/MRS procedure, the long acquisition time does not affect clinical routine. Our experimental setup is completely transferable to human applications, and can be fully integrated into the clinical workout of kidney transplantation from marginal donors.

## Conclusion

In this paper, we have shown that on kidneys presenting no WI, ATP analysis can be realized during  $O_2$ +HPP. The presence of enough  $O_2$  (100 kPa) is necessary for its resynthesis. Thanks to our new perfusion technology, it is possible to assess “in line” the ATP amount, which provides an absolute proof of cell viability. We have shown here the different energetic states of cold preserved organs taking in consideration HPP and Oxygenation. This technology may be used with any clinical MRI machine equipped with  $^{31}P$  spectroscopy and appears as a powerful tool to study various preservation conditions as well as warm ischemic effects.

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