Validation of Renal Oxidative Metabolism Measurement by Positron-Emission Tomography

Laurent Juillard, Sandrine Lemoine, Marc F. Janier, Paul Y. Barthez, Frédéric Bonnefoi, Maurice Laville

Abstract—Either in research or in clinical practice, the exploration of renal oxidative metabolism is limited by the lack of noninvasive measurement. Positron-emission tomography using carbon-11 acetate may estimate tissue oxidative metabolism by measuring acetate turnover in the Krebs cycle. Although extensively studied in cardiology, this method has never been validated for renal oxidative metabolism measurement. The aim of this study is the validation of acetate turnover compared with the invasive renal oxygen consumption measurement. Renal oxygen consumption and tubular sodium reabsorption were measured invasively in 10 anesthetized pigs. Simultaneously, acetate turnover was estimated by the clearance of carbon-11 acetate in the renal cortex, after a 166-MBq injection of carbon-11 acetate. Renal oxidative metabolism was measured under various conditions induced by mechanical and pharmacological interventions. Renal oxygen consumption and acetate turnover varied on a wide range from 0.05 to 0.29 mmol min\(^{-1}\) (>5-fold) and from 0.025 to 0.188 minutes\(^{-1}\) (>7-fold), respectively. Acetate turnover was very significantly correlated with renal oxygen consumption (\(P<0.0001; R=0.82\)) and tubular sodium reabsorption (\(P=0.001; R=0.67\)). This study demonstrates that acetate turnover measures renal oxidative metabolism noninvasively and quantitatively, consistent with changes in tubular sodium reabsorption. This method may be applied to assess oxidative metabolism in animal models and in humans. (Hypertension. 2007;50:242-247.)

Key Words: cell respiration ■ kidney ■ positron-emission tomography ■ carbon-11 acetate ■ oxygen consumption

Kidneys have the highest basal oxidative metabolism, representing 10% of basal body metabolism for 1% of the body weight. This very high oxidative metabolism is mainly involved in sodium reabsorption, and changes in renal oxidative metabolism correlate closely with changes in sodium reabsorption. It is, therefore, most likely that renal oxidative metabolism could be changed significantly in chronic renal diseases, ischemic nephropathy, or by drugs, such as diuretics.

Until now, changes in oxidative were studied by the measurement of oxygen consumption in isolated tubules or by the direct measurement of renal oxygen consumption using arteriovenous difference. Those methods are invasive and cannot be applied in humans. Therefore, the exploration of renal oxidative metabolism changes induced during human diseases is limited.

Positron-emission tomography (PET) is an imaging modality that provides quantitative and noninvasive measurements of functional parameters. After carbon-11–labeled acetate intravenous injection, renal cells extract acetate from the blood. Then, acetate is consumed in the Krebs cycle, depending on the intensity of oxidative metabolism, and is rejected in blood as \(^{13}\)CO\(_2\). Using this principle, acetate turnover measurement by PET has been extensively used in cardiology to evaluate heart viability. Shreve et al\(^6\) showed that renal acetate turnover measured by PET was markedly changed in human renal diseases. Importantly, carbon-11 acetate is not excreted by the kidney and will not accumulate in diseases that decrease renal function. Nevertheless, this method has never been validated experimentally. Moreover, because the energetic metabolism of kidneys and heart are markedly different, cardiac validation of PET acetate turnover may not be relevant for renal investigations. Therefore, the aim of this study was to validate renal oxidative metabolism measurement by PET with carbon-11 acetate turnover measurement using total renal oxygen consumption as the reference.

Methods

Animals
Ten 3- to 4-month–old female domestic pigs (Sus Scrofa) were used (29.8±0.4 kg). Animal care followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The institutional animal care and use committee approved the protocol.
**Renal Oxidative Metabolism Measurement by PET**

### Individual Interventions With Physiologic Parameters and Renal Oxygen Consumptions in Pigs at the Time of Acetate Turnover Measurements

<table>
<thead>
<tr>
<th>Pig/PET Measure</th>
<th>Interventions</th>
<th>Physiological Parameters and Renal Oxygen Consumptions</th>
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<tr>
<td></td>
<td>Interactions</td>
<td>Weight, kg</td>
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<tr>
<td></td>
<td>RAS</td>
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<td>Pig 1/1</td>
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<td>Pig 10/2</td>
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Interventions starting 30 min before PET measurements: RAS, renal artery stenosis induced to reduce renal blood flow to 60 to 80 mL min⁻¹; Furosemide (10 or 20 mg IV); Acetazolamide (500 mg IV); Dopamine (50 μg kg⁻¹ min⁻¹); and 20% hypertonic saline (20 mL IV over a 5 min period). Physiologic parameters: Weight (kg). NK—not known; MAP, mean arterial pressure (mm Hg); RBF, Renal blood flow (mL min⁻¹); GFR, glomerular filtration rate (mL min⁻¹); urine flow (mL min⁻¹); K mono, Acetate turnover (min⁻¹); Arterio venous oxygen consumption (mmol min⁻¹).

Pigs were sedated before anesthesia using 0.2 mg kg⁻¹ IM of droperidol (Droperil, Jansen-Cilag), 0.1 mg kg⁻¹ IM of xylazine (Remifentanil, Bayer Pharma), and 2 mg kg⁻¹ IM of ketamine (Ketalar, Parke-Davis). After tracheal intubation, anesthesia was maintained with a constant infusion of propofol (Diprivan, Zeneca Pharma) at 400 mg h⁻¹ (mean dose: 13 mg kg⁻¹ h⁻¹). An 8F catheter was inserted into the right internal carotid for arterial blood sampling and mean arterial pressure recording. A dual lumen catheter was inserted in the right internal jugular vein for isotonic saline (200 mL h⁻¹ during the whole experiment), inulin, and drug infusion. The right renal artery was dissected through a medial laparotomy. An ultrasonic flow probe (Transonic Systems Inc) was positioned around the proximal IV renal artery. In addition, a vascular occluder (Vascular Occluder, 3 mm, Harvard Apparatus) was positioned downstream from the flow probe in 3 animals. A 6F catheter was placed in the right renal vein through the right femoral vein. The right ureter was cannulated for urine collections. Then, animals were transported to the PET scanner room and installed in the scanner gantry.

### Protocol

To obtain a wide range of renal oxidative measurement, different combinations of maneuvers were performed (Table). Furosemide (10 or 20 mg IV; 6 pigs), acetazolamide (500 mg IV; 7 pigs), dopamine (50 μg kg⁻¹ min⁻¹ at constant rate; 7 pigs), and 20% hypertonic saline (20 mL IV over a 5-minute period; 4 pigs) were injected starting 30 minutes before PET measurement. The increase in urine flow after diuretic injection was not compensated by saline. Renal artery stenosis was induced (3 pigs) during the second PET measurement to decrease renal blood flow to 60 to 80 mL min⁻¹ starting 30 minutes before PET measurement. The interval between 2 PET acetate turnover measurements was 90 minutes.

### Acetate Turnover Measurement by PET

For each PET measurement, 166 MBq of carbon-11 acetate² were injected IV at a constant rate over 30 seconds. Starting simultaneously, a dynamic series of images was acquired using an HR+ PET scan (Siemens HR+, Siemens) with an average plane resolution of 5.5 mm for carbon-11 acetate, resulting in a 7- to 8-mm PET image resolution after reconstruction. Each series was composed of ten 10-second images, ten 20-second images, two 150-second images, and five 300-second images, corrected for tracer decay. For absolute quantification, photon attenuation was corrected using a transmission scan obtained with a ⁶⁷Ga source. Images were reconstructed using a filtered back projection method with a frequency cutoff of 0.4 mm⁻¹. For each slice, a static image (Figure 1a) was generated by summation of all of the dynamic images (Figure 1b). Regions of interest were drawn manually around the renal cortex on static images and copied on dynamic images to generate time activity curves for each renal cortex (Figure 2a), using the Median software (Université Catholique de Louvain).³ Time activity curves from each kidney cortex (~10 planes) were averaged (weighted by the number of pixels in the region of interest) to obtain a global cortical measurement comparable to the invasive measurement.

As described for cardiac studies, acetate turnover was measured with exponential fits, first with a mono exponential fit (Figure 2b), where the slope (Kmono) of the Ln of activity versus time estimates acetate turnover. Measurements included in this fit are the measurements from the rapid decreasing phase. Second is with a biexponential fit (Figure 2c):

\[
\text{acetate activity}(t) = ae^{-kt} + ce^{-kt}
\]

where \(k\) estimates acetate turnover.⁴ Measurements included in this fit are the measurements from the rapid and slow decreasing phase.

### Renal Function

Inulin (Inutest, Laevosan GmbH) was injected IV as an initial 10-minute bolus (30 mg kg⁻¹) followed by a constant rate infusion (0.33 mg kg⁻¹ min⁻¹). Inulin concentrations in plasma and urine were determined using a colorimetric method.⁵ Inulin clearance was calculated as \(C_i = \frac{V_i}{P_{ui}}\), where \(C_i\) is the urine concentration of inulin, \(V\) the volume of urine collected during each 20-minutes clearance period (starting 10 minutes before acetate injection), and \(P_{ui}\) is the plasma concentration of inulin. Sodium concentration in plasma and urine was measured with a flame photometer (IL943, Instrumentation Laboratory). Filtered sodium and tubular sodium reabsorption were calculated as \(P_{a,Na} \times GFR (P_{Na} \times GFR - U_{Na,Vi})\), respectively, where \(P_{a,Na}\) is the plasma concentration of sodium, \(GFR\)
is the glomerular filtration rate measured by inulin clearance, \(U_{\text{Na}}\) is the urine concentration of sodium, and \(V\) the volume of urine collected during each clearance period.

Oxygen consumption was calculated as the arteriovenous difference in oxygen concentration:

\[
\frac{\{(1.39 \times Hb \times S_{02}) + (0.03 \times P_{\text{O2}})\} \times RBF}{\{(1.39 \times Hb \times S_{02}) + (0.03 \times P_{\text{O2}})\} \times (RBF - U/F)}
\]

where \(Hb\) is the blood hemoglobin concentration, \(S_{02}\) is hemoglobin saturation in arterial blood, \(P_{\text{O2}}\) is oxygen partial pressure.
in arterial blood, $S_{O_2}$ is hemoglobin saturation in renal vein blood, $P_{vO_2}$ is oxygen partial pressure in renal vein blood measured with a blood gas analyser (Model 278, Ciba Corning), $RBF$ is renal blood flow measured by the ultrasound flow probe, and $UF$ is the urine flow.

**Calculations and Statistics**

Simple regressions were performed to describe the relationship between parameters. Differences were considered significant if $P<0.05$. Statistical analysis was performed using Statview (Abacus Concepts).

**Results**

Individual weight, mean arterial blood pressure, renal blood flow and glomerular filtration rate, and urine flow during PET acetate turnover measurement are summarized in the Table. Renal oxygen consumption varied on a wide range from 0.05 to 0.29 mmol min$^{-1}$ (Table). Acetate turnover varied also on a wide range, either measured with a monoexponential ($K_{mono}$: 0.025 to 0.188 minutes$^{-1}$; Table) or biexponential fitting ($K_1$: 0.054 to 0.225 minutes$^{-1}$). In the biexponential fit, the $K_2$ parameter was always estimated as 0. There was a very significant correlation between $K_{mono}$ and $K_1$ ($R=0.97$; $P<0.0001$).

Renal oxygen consumption correlated very significantly with $K_{mono}$ (Figure 4a; $R=0.82$; $P<0.0001$) and with $K_1$ ($R=0.72$). $K_{mono}$ and $K_1$ correlated significantly with filtered sodium ($R=0.672$, $P=0.001$ and $R=0.631$, $P=0.003$, respectively) and with tubular reabsorbed sodium...
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Although the correlation between acetate turnover and inva-
time, that is, 10 versus 30 minutes of acquisition, because late
redistribution justifying a more complex modeling. More-
significant for perfusion imaging, cost, and availability of tracers.

Discussion
This study demonstrates that PET using carbon-11 acetate
measures quantitatively renal oxidative metabolism and cor-
relates with the amounts of filtrated and reabsorbed sodium,
which are the physiological processes that affect renal oxygen
consumption primarily. Shreve et al\textsuperscript{6} were the first to show
that PET with carbon-11 acetate has the ability to detect
changes in oxidative metabolism in patients. As compared
with normal renal parenchyma, acetate turnover was reduced
in patients with chronic renal failure and dramatically
decreased in patients with renal cell carcinoma.

In our validation study, we tested the monoexponential and
biexponential fit described in cardiac studies.\textsuperscript{4,12,13} As op-
posed to cardiac studies,\textsuperscript{4,10} we found that invasive oxygen
metabolism was better correlated with the monoexponential
fit (R=0.82) than with the biexponential fit (R=0.72). In our
study, ce\textsuperscript{11} is always fitted as a constant, corresponding with a k2 equal to 0. This finding demonstrates that the decrease in
acetate activity in the kidney is monoexponential. This
discrepancy may be explained by differences in glutamate
metabolism in the heart and the kidney. In the heart, Arm-
brecht et al\textsuperscript{10} observed a rapid exponential clearance of tissue
activity after intracoronary injection of carbon-11 acetate,
reflecting turnover of acetate through the Krebs cycle, fol-
lowed by a slower clearance phase. Consistently, Ng et al\textsuperscript{14}
showed in another heart study that carbon-11 acetate was
trapped in a glutamate pool and that the slower phase after
carbon-11 acetate administration was because of the delayed
synthesis of glutamine and acetate from the glutamate pool.
Conversely, the glutamine in the kidney is oxidized by the
mitochondria of the proximal tubule in glutamate and NH4\textsuperscript{+},
providing the majority of ATP necessary for the reabsorption
of the sodium.\textsuperscript{15} Therefore, unlike in the myocardium, acetate
is not trapped in a glutamate pool, and there is no late
redistribution justifying a more complex modeling. More-
over, the monoexponential fit requires a shorter acquisition
time, that is, 10 versus 30 minutes of acquisition, because late
measurements are not included in the monoexponential fit.
Although the correlation between acetate turnover and inva-
sive oxygen consumption was very good, the correlation was
often higher in cardiac studies, pointing out some limitations of the reference method in the renal evaluation. First, we
compared the whole kidney oxygen consumption using the
arteriovenous difference with the acetate turnover measured
only in the cortex. Therefore, some discrepancies may occur
between the 2 measures, thus limiting the strength of the correl-
ation. Moreover, oxygen consumption is more homo-
genous in the myocardium than in the kidney, which
presents complex regional specific metabolic rates and path-
ways. Second, the time scale for measurements may intro-
duce some discrepancies: PET measurement last 10 minutes
when the invasive method measurement is punctual. Indeed,
the heart workload evaluated by the pressure rate product is
easier to clamp than the kidney workload evaluated retrospec-
tively by sodium reabsorption.

As shown earlier by Shreve et al,\textsuperscript{6} oxidative metabolism is
markedly changed in chronic disease. However, they showed
that acetate turnover was reproducible in either patients or
normal subjects.\textsuperscript{6} This point is critically important for mon-
toring intraindividual changes. The range of renal acetate
turnover reported by Shreve et al\textsuperscript{6} in normal subjects (0.11 to
0.16 minutes\textsuperscript{−1}) was similar to renal acetate turnover mea-
sured in animals without stimulation in our study. Impor-
tantly, our study demonstrates that acetate turnover may
monitor oxidative metabolism dynamically, because each
animal was studied accurately under 2 different experimental
conditions.

In our study, the different interventions performed created a
wide range of changes in oxygen consumption and acetate
turnover (>5 fold), reinforcing the validation of the method.
To have many conditions of oxygen consumption, we used
interventions that modified the sodium reabsorption (furo-
semide, acetazolamide, hypertonic saline, and artificial renal
artery stenosis). Indeed, we showed a correlation between the
acetate turnover and the reabsorption of sodium. However,
this correlation has less strength than with oxygen consump-
tion, because drugs, such as dopamine and diuretics, change
the ratio between oxygen consumption and sodium
reabsorption.\textsuperscript{16,17}

Pigs are commonly used in renal functional imaging studies for renal size and availability purpose. The cortex is 1
 cm thick, therefore, there is no need for partial volume
correction, especially with carbon-11 acetate, which provides
a good spatial resolution. As opposed to cardiac study,\textsuperscript{12} there is no spillover or need for correction of extrarenal arterial
contamination. However, as compared with human kidney,
pig kidneys are flat, and the medulla is thin. Therefore, it was
impossible to distinguish the medulla and evaluate medulla
metabolism, although Krebs cycle enzymes are also present
and active in the medulla.\textsuperscript{18} Anesthetic procedure that was
mandatory with animals may have changed the absolute
measurements of oxidative metabolism by deceasing sodium
excretion. However, the aim of the study was to validate the
method as compared with the invasive measurement of
oxygen consumption and not to provide physiological data.

The advantage of PET is to provide quantitative, single-
kidney, and noninvasive physiological parameter measure-
ments. Radionuclides are not nephrotoxic and are used at a
tracer concentration. Those advantages are balanced by irra-
diation, cost, and availability of tracers.

Perspectives
Functional imaging modalities, such as PET using oxygen
labeled water,\textsuperscript{19,20} electron beam computed tomography,\textsuperscript{21}
and potentially MRI,\textsuperscript{22} have the ability to quantitatively and
noninvasively monitor changes in renal perfusion. Indeed,
hemodynamic factors are triggering tissue damage through
different pathways in chronic renal diseases. To study meta-
oblic consequences of such damage, we think that renal
performance evaluation should include oxidative metabolism
measurement by acetate turnover and oxygen content evalu-
ation by MRI blood oxygenation level–dependent contrast.
MRI blood oxygenation level–dependent contrast is an emerging tool for measuring renal oxygen content.23,24 The concomitant measurements of perfusion, oxygen content, and consumption would help in monitoring the effects of interventions and in understanding the pathophysiology of chronic renal diseases, such as ischemic nephropathy.

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Disclosures
None.

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