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 (ρ<0.0001; R=0.82) and tubular sodium reabsorption (ρ=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.¹ This very high oxidative metabolism is mainly involved in sodium reabsorption, and changes in renal oxidative metabolism correlate closely with changes in sodium reabsorption.²⁻³ 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.³ 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 ¹¹CO₂.⁴ Using this principle, acetate turnover measurement by PET has been extensively used in cardiology to evaluate heart viability.⁵ Shreve et al⁶ 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.⁶ 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.
Juillard et al  Renal Oxidative Metabolism Measurement by PET 243

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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RAS</td>
<td>MAP, mm Hg</td>
</tr>
<tr>
<td>Pig 1/1</td>
<td>X</td>
<td>28.5</td>
</tr>
<tr>
<td>Pig 1/2</td>
<td>X, X</td>
<td>51</td>
</tr>
<tr>
<td>Pig 2/1</td>
<td>X, X</td>
<td>29.5</td>
</tr>
<tr>
<td>Pig 2/2</td>
<td>X, X</td>
<td>99</td>
</tr>
<tr>
<td>Pig 3/1</td>
<td>X, X</td>
<td>31</td>
</tr>
<tr>
<td>Pig 3/2</td>
<td>X, X</td>
<td>84</td>
</tr>
<tr>
<td>Pig 4/1</td>
<td>X, X</td>
<td>30.4</td>
</tr>
<tr>
<td>Pig 4/2</td>
<td>X, X</td>
<td>98</td>
</tr>
<tr>
<td>Pig 5/1</td>
<td>X, X</td>
<td>84</td>
</tr>
<tr>
<td>Pig 5/2</td>
<td>X, X</td>
<td>90</td>
</tr>
<tr>
<td>Pig 6/1</td>
<td>X, X</td>
<td>27.5</td>
</tr>
<tr>
<td>Pig 6/2</td>
<td>X, X</td>
<td>74</td>
</tr>
<tr>
<td>Pig 7/1</td>
<td>X, X</td>
<td>31.2</td>
</tr>
<tr>
<td>Pig 7/2</td>
<td>X, X</td>
<td>91</td>
</tr>
<tr>
<td>Pig 8/1</td>
<td>X, X</td>
<td>30.3</td>
</tr>
<tr>
<td>Pig 8/2</td>
<td>X, X</td>
<td>61</td>
</tr>
<tr>
<td>Pig 9/1</td>
<td>X, X</td>
<td>103</td>
</tr>
<tr>
<td>Pig 9/2</td>
<td>X, X</td>
<td>67</td>
</tr>
<tr>
<td>Pig 10/1</td>
<td>X, X</td>
<td>67</td>
</tr>
<tr>
<td>Pig 10/2</td>
<td>X, X</td>
<td>78</td>
</tr>
</tbody>
</table>

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 (Droperilan, Janssen-Cilag), 0.1 mg kg⁻¹ IM of xylazine (Kompun, Bayer Pharma), and 2 mg kg⁻¹ IM of ketamine (Ketalan, Parke-Davis). After tracheal intubation, anesthesia was maintained with a constant infusion of propofol (Diprivan, Zeneca Pharma) (0.33 mg kg⁻¹ h⁻¹). 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 left 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,⁶ resulting in a 7- to 8-mm PET image resolution after reconstruction. Each series was composed of ten 1-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 ⁶⁸Ge 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 Mediman 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):

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

where \(k\) estimates acetate turnover.¹⁰ 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.¹¹ Inulin clearance was calculated as \(U_{in} \times VIP_{urin}\), where \(U_{in}\) 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 \(P_{in}\) 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 \(P_{Na} \times GFR\) and \(P_{Na} \times GFR - U_{Na}/V\), respectively, where \(P_{Na}\) 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:

\[
\text{Oxygen consumption} = \left\{ \frac{\left( 1.39 \times \frac{Hb}{Hb} \times S_aO_2 \right) - 0.03 \times P_{O_2}}{RBF} \right\} \times \left( \frac{RBF}{U} \right)
\]

(2)

where \( Hb \) is the blood hemoglobin concentration, \( S_aO_2 \) is hemoglobin saturation in arterial blood, \( P_{O_2} \) is oxygen partial pressure.

Figure 1. A. Renal PET static images using carbon-11 acetate in pigs. Each image represents a transverse slice where the kidneys are displayed (orientation: Ant=up; Right=left; the top left image represents the subdiaphragm slice, the lower right images represents the inferior slice). B. Renal PET dynamic images using carbon-11 acetate in pigs. Each image represents the evolution of acetate activity in the aorta and kidneys at different times after carbon-11 acetate (as described in the Methods section), corresponding with slice 6 of the static image. Orientation: Ant=up; Right=left; the top left image represents the initial frame, the lower right images represents last frame. Note residual increased activity on the right kidney downstream a renal artery stenosis (stenotic and contra lateral kidneys are labeled in plane 6).
in arterial blood, $S_vO_2$ 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 $K_1$ (Figure 3; $P<0.0001$; $R=0.97$).

Renal oxygen consumption correlated very significantly with $K_{mono}$ (Figure 4a; $P<0.0001$; $R=0.82$) and with $K_1$ ($P<0.0003$; $R=0.72$). $K_{mono}$ 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.
the heart workload evaluated by the pressure rate product is when the invasive method measurement is punctual. Indeed, the monoexponential fit described in cardiac studies. As opposed to cardiac studies, 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, \( ce^{-k_2t} \) is always fitted as a constant, corresponding with a \( k_2 \) 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, Armbricht et al observed a rapid exponential clear-\(^{\text{in}}\) ace activity after intracoronary injection of carbon-11 acetate, reflecting turnover of acetate through the Krebs cycle, followed by a slower clearance phase. Consistently, Ng et al 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 \( \text{NH}_4^+ \), providing the majority of ATP necessary for the reabsorption of the sodium. Therefore, unlike in the myocardium, acetate is not trapped in a glutamate pool, and there is no late redistribution justifying a more complex modeling. Moreover, 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 invasive 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 correlation. Moreover, oxygen consumption is more homogeneous in the myocardium than in the kidney, which presents complex regional specific metabolic rates and pathways. Second, the time scale for measurements may introduce 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 retrospectively by sodium reabsorption.

As shown earlier by Shreve et al, oxidative metabolism is markedly changed in chronic disease. However, they showed that acetate turnover was reproducible in either patients or normal subjects. This point is critically important for monitoring intraindividual changes. The range of renal acetate turnover reported by Shreve et al in normal subjects (0.11 to 0.16 minutes \(^{-1} \)) was similar to renal acetate measured in animals without stimulation in our study. Importantly, 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 (furosemide, 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 consumption, because drugs, such as dopamine and diuretics, change the ratio between oxygen consumption and sodium reabsorption.

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, 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. 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 measurements. Radionuclides are not nephrotoxic and are used at a tracer concentration. Those advantages are balanced by irradiation, cost, and availability of tracers.

**Perspectives**

Functional imaging modalities, such as PET using oxygen labeled water, electron beam computed tomography, and potentially MRI, 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 metabolic consequences of such damage, we think that renal performance evaluation should include oxidative metabolism measurement by acetate turnover and oxygen content evaluation by MRI blood oxygenation level–dependent contrast.
MRI blood oxygenation level–dependent contrast is an emerging tool for measuring renal oxygen content.\textsuperscript{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.

Acknowledgments

We thank Prof Gérard Gimenez, director of Centre D’exploration et de Recherche Médicale par Emission de Positons, and his staff for support in this endeavor.

Sources of Funding

This work was supported by INSERM ERI22, Université Claude Bernard Lyon 1, Hospices Civils de Lyon, and École Nationale Vétérinaire De Lyon.

Disclosures

None.

References

Validation of Renal Oxidative Metabolism Measurement by Positron-Emission Tomography

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

Hypertension. 2007;50:242-247; originally published online May 14, 2007; doi: 10.1161/HYPERTENSIONAHA.107.089607

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/50/1/242

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/