[\(^{13}\text{C}\)]ACETATE CLEARANCE AS AN
INDEX OF OXYGEN CONSUMPTION
OF THE RIGHT MYOCARDIUM IN
IDIOPATHIC PULMONARY ARTERIAL
HYPERTENSION: A VALIDATION
STUDY USING OXYGEN-15 LABELED
TRACERS AND PET

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Submitted
ABSTRACT

Idiopathic pulmonary arterial hypertension (IPAH) results in increased right ventricular (RV) workload and oxygen demand. Previously, it has been shown that myocardial oxygen consumption (MVO₂) of the hypertrophied RV of IPAH patients can be measured using PET and oxygen-15 labeled tracers. This method, however, is less suitable for routine clinical practice. The purpose of the present study was to assess whether MVO₂ could also be determined in the RV of IPAH patients from the clearance of [¹¹C]acetate, a simple method that is in use for MVO₂ measurements of the left myocardium.

Methods

Seventeen IPAH patients underwent four consecutive PET scans. [¹⁵O]H₂O, [¹⁵O]O₂ and [¹⁵O]CO scans were used to derive RV flow, oxygen extraction fraction and blood volume, respectively, from which RV MVO₂ was calculated. The rate of clearance Kmono and the efflux rate constant k₂ were derived from the [¹¹C]acetate scan. The RV rate pressure product (RPP) was also determined by means of right heart catherization, as an index of the RV MVO₂ and was calculated as the product of systolic pulmonary artery pressure and heart rate.

Results

For the total population, both Kmono (r=0.64, P=0.006) and k₂ (r=0.67, P=0.003) showed a significant correlation with RV MVO₂. They also correlated with RV RPP, Kmono: r=0.74, p < 0.001; k₂: r=0.70, p = 0.002.

Conclusion

Both measures of [¹¹C]acetate clearance were significantly correlated with quantitative RV MVO₂ measurements in IPAH patients, indicating that they can both be used as an index of RV oxygen metabolism.

Keywords

[¹¹C]acetate, oxygen-15, PET, mono-exponential curve fitting, compartmental modelling, pulmonary arterial hypertension, right ventricle, myocardial oxygen consumption
INTRODUCTION

The oxygen consumption ($MVO_2$) of the normal right myocardium is less than that of the left heart as a result of a lower afterload. In idiopathic pulmonary arterial hypertension (IPAH), elevated pulmonary vascular resistance causes right ventricular (RV) hypertrophy and ultimately fatal RV failure. To gain insight into the pathophysiology of a reduced pump function of the failing heart, quantification of $MVO_2$ is important. A noninvasive method using state-of-the-art positron emission tomography (PET) and oxygen-15 labelled tracers has been validated as a means to measure $MVO_2$ accurately. Using this method, we show in Chapter 7 that the RV of severe IPAH patients consumes more oxygen for generating similar RV power output as in mild IPAH. This method is, however, extensive and time-consuming, requiring an on-site cyclotron to produce the short-lived oxygen-15 tracers, and three consecutive PET scans ($[^{15}O]H_2O$, ($[^{15}O]$ CO) and $[^{15}O]O_2$). PET using $[^{13}C]$acetate has been proposed as a more practical method to estimate $MVO_2$. Being the precursor of acetyl-CoA in the tricarboxylic acid (TCA) cycle, the rate of $[^{13}C]$acetate clearance determined by mono-exponential curve fitting ($K_{mono}$), is closely related to $MVO_2$ of the left heart.

It is, however, not known whether $[^{13}C]$acetate can also be used to estimate RV $MVO_2$. If so, the use of $[^{13}C]$acetate-PET would make future studies on oxidation in the failing right ventricle more convenient. Recently it was shown that a single compartment model efflux rate constant ($k_j$) correlated better with the rate pressure product (RPP), a non-invasive index of myocardial oxygen demand, than $K_{mono}$. However, studies performing a direct comparison of $MVO_2$ and different methods of $[^{13}C]$acetate clearance determination in the human right ventricle are lacking. Therefore, the purpose of the present study was to assess whether the RV clearance rates of $[^{13}C]$acetate correlate with RV RPP and the $MVO_2$ (in units) of the hypertrophied right ventricle in IPAH patients.

METHODS

$[^{13}C]$acetate clearance – an index of RV $MVO_2$ in IPAH

The present study is part of a larger investigation of the oxidative metabolism of the hypertrophied right ventricle in IPAH patients, which was approved by the Medical Ethics Review Committee of the VU University Medical Center. In total, 17 patients (16 females) were recruited between April 2008 and October 2010. All had IPAH according to WHO group I criteria with either NYHA class II or III. Sixteen patients had clinically stable IPAH under optimal treatment. The change in six-minute walk distance was less than 10% within 6 months prior to inclusion. During the study, PAH therapy was continued, which was either single or combination treatment of intravenous prostacyclines, endothelin receptor antagonists or phosphodiesterase-type 5 inhibition. One patient was newly diagnosed with IPAH NYHA class III and included prior to treatment. Exclusion criteria were cardiovascular disease, atrial fibrillation and anaemia (haemoglobin <12g/dL). All patients gave written informed consent prior to inclusion in the study. A control group was not included because the normal right myocardium is too thin to be measured reliably using PET and oxygen-15 tracers.
PET scanning protocol

All patients underwent consecutive $[^{15}O]H_2O$, $[^{15}O]O_2$, $[^{15}O]CO$, and $[^{11}C]acetate$ scans under resting conditions using an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, TN, USA). After a light breakfast, 2 hours prior to the first scan, patients fasted until the end of the scanning protocol. One canula was inserted in an antecubital vene for tracer administration and another in the radial artery for arterial blood sampling. After positioning the patient on the scanner bed, a 15 minutes transmission scan was performed, which was used to correct all subsequent emission scans for photon attenuation. Next, a 10 minutes dynamic emission scan (40 frames) was acquired in 2D acquisition mode, following an intravenous injection of 1.1 GBq $[^{15}O]H_2O$. After an interval of 10 minutes to allow for decay of oxygen-15, a second identical emission scan was acquired following a bolus inhalation of 7 GBq $[^{15}O]O_2$ Next, one min after the end of a bolus inhalation of 4 GBq $[^{15}O]CO$, a 6 minutes ECG-gated static emission scan was acquired. After a two-hours break, the patient was repositioned on the scanner bed and a second 10 minutes transmission scan was acquired. Finally, following an intravenous injection of 300 MBq $[^{11}C]acetate$ (48 min and 29 frames), a 48 minutes dynamic emission scan (29 frames) was acquired in 3D-acquisition mode. Systemic blood pressure, peripheral saturation and heart rate were monitored at set intervals during the protocol.

Data analysis

Analysis of the oxygen-15 data has been described previously. For the present study, the region of interest (ROI) was the RV free wall, excluding the septum. In brief, an anatomical tissue fraction (ATF) image was generated by subtracting the blood pool image (derived from the $[^{15}O]CO$ scan) from the transmission image. This ATF image was resliced into short axis images according to the anatomic axes of the left ventricle. The same reslicing parameters were applied to both dynamic $[^{15}O]H_2O$ and $[^{15}O]O_2$ images. Using the ATF image, an RV free wall region of interest (ROI) was defined and projected onto dynamic $[^{15}O]H_2O$ and $[^{15}O]O_2$ images to generate time-activity curves. RV MBF was determined from the $[^{15}O]H_2O$ time-activity curve using the standard single tissue compartment model. RV OEF was determined from the $[^{15}O]O_2$ time-activity curve using a recent implementation of a model described previously, in which RV MBF, perfusible tissue fraction, arterial blood volume and RV spill-over were fixed to values determined from the $[^{15}O]H_2O$ fit and in which a correction for spill-over from activity in the pulmonary gas volume was applied as described previously. Both $[^{15}O]H_2O$ and $[^{15}O]O_2$ input function were based on a volume of interest drawn in the ascending aorta. The $[^{15}O]O_2$ input function was corrected for the contribution of recirculating water derived from the arterial blood samples.

The $[^{11}C]acetate$ data underwent a similar procedure, except that a summed $[^{11}C]acetate$ image (between 3rd and 5th minute) was used to reslice the dynamic scan and to define the RV free wall ROI. As described previously, the $[^{11}C]acetate$ time-activity curve was analysed by fitting the clearance phase to a single exponential, providing the clearance rate $K_{\text{mono}}$ (min$^{-1}$). In addition, the entire $[^{11}C]acetate$ time-activity curve was fitted to a single tissue compartment model, providing the rate constant $k_2$ (min$^{-1}$) for transfer of $[^{11}C]acetate$ from tissue to blood after correction for labeled metabolites.
Right ventricular haemodynamics

RV haemodynamics were obtained during right heart catheterisation and cardiac MRI within one week of the PET study when possible, according to procedures described previously. In three subjects, for logistical or personal reasons, the interval between RHC and PET was 20 to 55 days, and between cMRI and PET 20 to 36 days. As these patients had stable IPAH under drug treatment, the interval was considered acceptable. RV RPP, an index of global ventricular work and thus of myocardial oxygen demand, is defined as the product of systolic pulmonary arterial pressure and heart rate.

Statistical analyses

All results are expressed as mean ± SD. Linear regression analysis was used (Prism 5 for Windows, GraphPad Software, San Diego, CA). P<0.05 was considered statistically significant.

RESULTS

Patient characteristics and haemodynamics are shown in Table 4.1. Table 4.2 summarizes RPP and PET derived measures of RV oxygen consumption. There was a significant correlation between $K_{\text{mono}}$ and $\text{MVO}_2$ ($r=0.64$, $p = 0.006$; Figure 4.1A). A similar correlation was found between $k_3$ and RV $\text{MVO}_2$ ($r=0.67$, $p = 0.003$; Figure 4.1B). $K_{\text{mono}}$ and $k_3$ also correlated significantly with RPP ($K_{\text{mono}}$: $r=0.74$, $p < 0.001$; $k_3$: $r=0.70$, $p = 0.002$; Figures 4.2A and B). The treatment-naïve IPAH patient lies in the upper right corner in Figures 4.1 and 4.2. The significance of the correlations between clearance rates and RV $\text{MVO}_2$ remained after exclusion of this patient ($K_{\text{mono}}$: $r=0.54$, $p = 0.029$; $k_3$: $r = 0.58$, $p = 0.019$). However, the correlations with RPP became non-significant after exclusion of this patient (both $p = 0.10$).

Table 4.1 Clinical characteristics and haemodynamics at time of inclusion.

<table>
<thead>
<tr>
<th>Clinical characteristic</th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td>NYHA class II / III</td>
<td>8 / 9</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>45 ± 12</td>
</tr>
<tr>
<td>Mean RAP (mmHg)</td>
<td>8 ± 7</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>9 ± 3</td>
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<tr>
<td>Mean PAP (mmHg)</td>
<td>53 ± 15</td>
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<tr>
<td>Cardiac output (L · min⁻¹)</td>
<td>5.1 ± 1.4</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>76 ± 14</td>
</tr>
<tr>
<td>PVR (dyne · s · cm⁻²)</td>
<td>730 ± 335</td>
</tr>
<tr>
<td>RV ejection fraction (%)</td>
<td>34 ± 17</td>
</tr>
<tr>
<td>6MWD (m)</td>
<td>458 ± 112</td>
</tr>
<tr>
<td>Nt-proBNP (ng · L⁻¹)</td>
<td>1368 ± 1794</td>
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</table>

6MWD six-minute walk distance; Nt-ProBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; PAP, pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; RAP, right atrial pressure.
**Table 4.2** Rate pressure product and PET derived measures of right ventricular oxygen consumption

<table>
<thead>
<tr>
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<th>Mean ± SD</th>
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<tbody>
<tr>
<td>RV RPP (mmHg · bpm)</td>
<td>6610 ± 2620</td>
</tr>
<tr>
<td>MVO₂ (ml · min⁻¹ · g⁻¹ right myocardium)</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>Kmono (min⁻¹)</td>
<td>0.058 ± 0.015</td>
</tr>
<tr>
<td>K₂ (min⁻¹)</td>
<td>0.099 ± 0.024</td>
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</table>

*Kmono*, mono-exponential rate of clearance of [¹¹C]acetate; *k₂*, tissue to plasma efflux rate constant of [¹¹C]acetate; MVO₂, myocardial oxygen consumption derived from [¹⁵O]-PET; RPP, rate pressure product obtained from systolic pulmonary artery pressure and heart rate; RV, right ventricular.

**Figure 4.1** (A) Mono-exponential rate of clearance Kmono and (B) tissue to plasma efflux rate constant of [¹¹C]acetate as function of RV myocardial oxygen consumption (MVO₂).

**Figure 4.2** (A) Mono-exponential rate of clearance Kmono and (B) tissue to plasma efflux rate constant of [¹¹C]acetate as function of rate pressure product (RPP).

**DISCUSSION**

The main findings of this study were that the rate of clearance of [¹¹C]acetate correlated, although weakly, with both MVO₂ as measured using PET and oxygen-15 labelled tracers, and RPP in the hypertrophied RV wall of IPAH patients, with little difference between Kmono and k₂. The correlations with MVO₂ remained after exclusion of the treatment-naïve patient, but those with RPP were lost.
The significant correlation between $[11C]$acetate clearance rate $K_{\text{mono}}$ and $MVO_2$ in the hypertrophied right ventricle is in agreement with previous studies on the left myocardium in both control subjects ($r=0.71, p=0.02$) and patients with chronic myocardial infarction ($r=0.89, p<0.001$).\textsuperscript{4, 7-10} Little is known, however, about the rate of clearance of $[11C]$acetate from the normal right ventricle. Two previous studies reported a mean $K_{\text{mono}}$ value of 0.040 to 0.043 min$^{-1}$ in the normal right ventricle of control subjects, which was significantly lower than values seen in patients with dilated cardiomyopathy.\textsuperscript{9, 19} The latter values (0.050 - 0.055 min$^{-1}$) are similar to the values observed in the present study. It has been proposed that the higher clearance rate of $[11C]$acetate is likely due to an increased RV workload in LV heart failure, i.e. due to secondary pulmonary hypertension. Accordingly, high RV $K_{\text{mono}}$ values in the present population of IPAH patients indeed reflect increased oxygen demand of the right myocardium as the result of increased afterload in IPAH. In line with that, the treatment-naïve IPAH patient had the highest RV $K_{\text{mono}}$, $k_2$ and $MVO_2$ values, indicative for a high RV oxidative metabolism as a result of untreated IPAH.

In contrast to the findings of Timmer et al.\textsuperscript{7} for the normal left ventricle, the correlation between $k_2$ and RV RPP was not superior to that of $K_{\text{mono}}$ and RV RPP. In fact, for both $k_2$ and $K_{\text{mono}}$ the correlation was largely based on the treatment-naïve patient as the outlier. It disappeared when the outlier was excluded. A possible explanation for the lack of correlation is that RV RPP and $[11C]$acetate-PET were not measured on the same day, unlike the oxygen-15-PET and $[11C]$acetate-PET. It can also be due to the fact that the RV RPP is only an index for global $MVO_2$.\textsuperscript{11} Correlation with another index of the oxidative metabolism, i.e. the acetate clearance rate, results in loss of relation when the range in RV RPP is too small among the study population after exclusion of the treatment-naïve patient (Figure 4.2).

The golden standard to obtain myocardial oxygen consumption is via invasive Fick measurement. However, this is not possible for the right ventricle as it lacks a common venous drainage like the coronary sinus. The recently introduced noninvasive approach using oxygen-15 labeled tracers and PET resulted in reproducible RV oxygen extraction values and thus reliable quantification of RV $MVO_2$.\textsuperscript{14} The present correlations of $[11C]$acetate derived $K_{\text{mono}}$ and $k_2$ with quantitative RV $MVO_2$ suggest that $[11C]$acetate clearance rate can be used as an index of RV oxidative metabolism in IPAH. However, $[11C]$acetate cannot be used for quantification of $MVO_2$ in absolute units, especially as acetate clearance can be affected by metabolic shifts in remodeling RV myocardium in IPAH. The latter is based on the fact that, in failing myocardium, other processes may cause uncoupling of oxygen consumption and ADP phosphorylation, e.g. increased production of NO and reactive oxygen species.\textsuperscript{20} Therefore, the assumed tight coupling of the rate of clearance of acetate via the TCA to oxygen handling in the respiratory chain as found in the normal heart may lack in diseased myocardium. In such cases, $[11C]$acetate clearance would overestimate ATP synthesis.

For the left myocardium in the present study population there was no correlation found between $[11C]$acetate clearance rate and $MVO_2$ (Table S4.1 and Figure S4.1).

Limitations

A measurement error in the data may have been introduced as a result of the scanning protocol being performed not simultaneously but in two sessions on the same day. The patient position in the scan may not be exactly the same in the two sessions. Furthermore, it cannot be excluded
that the myocardial metabolism may alter during the day, as the 15O PET is performed 2 hours following a light meal whereas the [11C]acetate PET is obtained about 6 hours later. The protocol was, however, too extensive to demand the patients to fast from midnight before the day of scanning till the end of the PET study. The protocol was standardized as much as possible. The measurement of the pulmonary artery pressure and heart rate for the calculation of RPP was performed on another day, which may also have contributed to error. Finally, we have only focused on myocardial metabolic measurements at resting conditions. It is possible that more solid correlations would appear when exercise or inotropic drugs had been used to obtain a larger range of data. However, the demanding study protocol and the severity of the disease, did not allow strenuous exercise or medical interventions.

**Conclusion**

Clearance of [11C]acetate, expressed as either $K_{m00}$ or $k_2$, significantly correlated with quantitative RV MVO$_2$ measurements in hypertrophied right myocardium of IPAH patients. Therefore, [11C] acetate PET holds promise as a method to monitor changes in RV oxygen metabolism following therapeutic interventions in IPAH studies.
REFERENCE LIST


SUPPLEMENTARY DATA

Data of left ventricular (LV) MVO₂ and [¹¹C]acetate clearance rate in the present study population are shown in Table S4.1 and Figure S4.1A,B,C,D. There was no correlation found between [¹¹C]acetate clearance rate and MVO₂ in the left myocardium in IPAH patients nor was there a correlation with LV RPP. This may be due to the limited range of these parameters under resting conditions, since the LV afterload in IPAH is not affected. Moreover, a recent study shows that the left ventricle is atrophic due to unloading and reduced cardiac output in RV failure secondary to chronic thromboembolic pulmonary hypertension.¹ It is possible that this reduced LV preload is also present in IPAH resulting in a level of LV oxidative metabolism with little variance in the study group; such that the range of the estimated MVO₂ expressed as [¹¹C]acetate clearance rate, is then too insensitive to achieve specific relation with quantitative MVO₂ of the left myocardium.

Table S4.1 Rate pressure product and PET derived measures of right ventricular oxygen consumption

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td>LV RPP (mmHg · bpm)</td>
<td>8605 ± 1926</td>
</tr>
<tr>
<td>MVO₂ (ml · min⁻¹ · g⁻¹ left myocardium)</td>
<td>0.10 ± 0.03</td>
</tr>
<tr>
<td>LV K_{mono} (min⁻¹)</td>
<td>0.063 ± 0.011</td>
</tr>
<tr>
<td>LV k₂ (min⁻¹)</td>
<td>0.099 ± 0.024</td>
</tr>
</tbody>
</table>

K_{mono}, mono-exponential rate of clearance of [¹¹C]acetate; k₂, tissue to plasma efflux rate constant of [¹¹C]acetate; LV, left ventricular; MVO₂, myocardial oxygen consumption derived from [¹⁵O]-PET; RPP, rate pressure product obtained from systolic blood pressure and heart rate.
Figure S4.1 Correlations of MVO2 (panels A, B) or LV RPP (panels C, D) with [11C]acetate clearance rates monoexponential rate of clearance of [11C]acetate (Kmono) and tissue to plasma efflux rate constant of [11C]acetate (k2) in the left myocardium of IPAH patients. There was no significant correlation found.

SUPPLEMENTARY REFERENCE LIST