Heterogeneity in Lung 18FDG Uptake in Pulmonary Arterial Hypertension

Potential of Dynamic 18FDG Positron Emission Tomography With Kinetic Analysis as a Bridging Biomarker for Pulmonary Vascular Remodeling Targeted Treatments

Lan Zhao, MD, PhD; Ali Ashek, PhD; Lei Wang, MD; Wei Fang, MD, PhD; Swati Dabral, PhD; Olivier Dubois, BScEd; John Cupitt, PhD; Soni Savai Pullamsetti, PhD; Emanuele Cotroneo, PhD; Hazel Jones, PhD; Gianpaolo Tomasi, PhD; Quang-De Nguyen, PhD; Eric O. Aboagye, PhD; Mona A. El-Bahrawy, PhD; Gareth Barnes, MD; Luke S. Howard, DPhil; J. Simon R. Gibbs, MD; Willy Gsell, PhD; Jian-Guo He, MD, PhD; Martin R. Wilkins, MD

Background—Pulmonary arterial hypertension (PAH) is a disease of progressive vascular remodeling, characterized by dysregulated growth of pulmonary vascular cells and inflammation. A prevailing view is that abnormal cellular metabolism, notably aerobic glycolysis that increases glucose demand, underlies the pathogenesis of PAH. Increased lung glucose uptake has been reported in animal models. Few data exist from patients with PAH.

Methods and Results—Dynamic positron emission tomography imaging with fluorine-18–labeled 2-fluoro-2-deoxyglucose (18FDG) ligand with kinetic analysis demonstrated increased mean lung parenchymal uptake in 20 patients with PAH, 18 with idiopathic PAH (IPAH) (FDG score: 3.27±1.22), and 2 patients with connective tissue disease (5.07 and 7.11) compared with controls (2.02±.71; P<0.05). Further compartment analysis confirmed increased lung glucose metabolism in IPAH. Lung 18FDG uptake and metabolism varied within the IPAH population and within the lungs of individual patients, consistent with the recognized heterogeneity of vascular pathology in this disease. The monocrotaline rat PAH model also showed increased lung 18FDG uptake, which was reduced along with improvements in vascular pathology after treatment with dichloroacetate and 2 tyrosine kinase inhibitors, imatinib and sunitinib. Hyperproliferative pulmonary vascular fibroblasts isolated from IPAH patients exhibited upregulated glycolytic gene expression, along with increased cellular 18FDG uptake; both were reduced by dichloroacetate and imatinib.

Conclusions—Some patients with IPAH exhibit increased lung 18FDG uptake. 18FDG positron emission tomography imaging is a tool to investigate the molecular pathology of PAH and its response to treatment. (Circulation. 2013;128:1214-1224.)

Key Words: biomarkers • glucose metabolism • hypertension, pulmonary • imaging

Pulmonary arterial hypertension (PAH) is a disease of progressive vascular remodeling characterized by dysregulated growth of pulmonary vascular cells and inflammation. In the search for new medicines, the focus is moving toward therapies targeting these mechanisms, drawing on approved oncology interventions, such as tyrosine kinase inhibitors (eg, imatinib)5,6 and metabolic modulators (eg, dichloroacetate),7,8 and anti-inflammatory treatments.9 The evaluation of these antiremodeling and anti-inflammatory strategies in PAH patients poses a considerable challenge that may hamper their development.

Received March 26, 2013; accepted July 12, 2013.
From the Centre for Pharmacology and Therapeutics, Experimental Medicine, Imperial College London, Hammersmith Hospital, London, UK (L.Z., A.A., L.W., O.D., J.C., E.C., H.J., G.B., M.R.W.); Department of Nuclear Medicine, Cardiovascular Institute and Fu Wai Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China (W.F.); Max-Planck Institute for Heart and Lung Research and University of Giessen and Marburg Lung Center, Bad Nauheim, Germany (S.D., S.S.P.); Comprehensive Cancer Imaging Centre, Department of Surgery and Cancer, Imperial College London, Hammersmith Hospital, London, UK (G.T., Q.N., E.O.A.); Department of Histopathology, Imperial College London, UK (M.A.E.-B.); National Heart and Lung Institute, Imperial College London, and National Pulmonary Hypertension Service, Department of Cardiology, Hammersmith Hospital, London, UK (L.S.H., J.S.R.G.); Biological Imaging Centre, Medical Research Council Clinical Sciences Centre, Imperial College London, Hammersmith Hospital, London, UK (W.G.); and Center for Diagnosis and Management of Pulmonary Vascular Diseases, Department of Cardiology, Cardiovascular Institute and Fu Wai Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China (J.H.).

The online-only Data Supplement is available with this article at http://circ.ahajournals.org/lookup/suppl/doi:10.1161/CIRCULATIONAHA.113.004136/-/DC1.

Correspondence to Lan Zhao, MD, PhD, Centre for Pharmacology and Therapeutics, Experimental Medicine, Imperial College London, Du Cane Road, London W12 0NN, UK. E-mail l.zhao@imperial.ac.uk

© 2013 American Heart Association, Inc.

Circulation is available at http://circ.ahajournals.org DOI: 10.1161/CIRCULATIONAHA.113.004136

1214
Noninvasive molecular imaging with the use of positron emission tomography (PET) offers enormous potential for monitoring cellular and biochemical events in otherwise inaccessible tissue and has been used in oncology to assess antiproliferative therapeutics. Specifically, fluorine-18–labeled 2-fluoro-2-deoxyglucose (18FDG), a glucose analogue, is widely used for the detection and staging of a variety of malignant lesions and can provide a quantitative assessment of response to treatment.10–13 18FDG PET exploits the "Warburg effect,"14,15 the observation that many cancers use aerobic cytoplasmic glycolysis as opposed to mitochondrial glucose oxidation as a major energy source, a process that requires increased cellular glucose uptake.

Aerobic glycolysis is also a characteristic of nonmalignant proliferating cells and is observed in human pulmonary endothelial cells isolated and cultured from idiopathic PAH (IPAH) patient lungs,16 as well as in pulmonary arterial smooth muscle cells from rodent PAH models.17 Lung parenchymal glucose uptake, measured by 18FDG PET, has been reported to be increased in IPAH patients compared with healthy controls.16,18 Recently, Marsboom et al19 showed an increased lung 18FDG PET signal in animal PAH models that is reduced by treatment with imatinib and dichloroacetate. These data provide the foundation for further investigation of the utility of 18FDG PET as a tool in the assessment of patients with PAH.

We set out to explore 3 questions: (1) the utility of dynamic 18FDG PET acquisition in discriminating between PAH patients and healthy controls; (2) the feasibility of 18FDG PET in tracking the pathology of pulmonary hypertension in in vivo PAH models and their response to treatment; and (3) the impact of treatments on cellular 18FDG uptake with the use of IPAH-derived cells in vitro. Our data demonstrate interindividual and intraindividual variability in 18FDG uptake in IPAH lungs. 18FDG PET may offer a tool for deep phenotyping IPAH patients to understand the response to new PAH-targeted treatments in a clinical trial.

### Methods

#### Patient Population

Twenty patients (Table 1) diagnosed with PAH by standard criteria20 attending Hammersmith Hospital, London (10 patients with IPAH), and Fuwai Hospital, Beijing (8 patients with IPAH and 2 with connective tissue disease), from April 2011 to December 2012, as well as 5 nonsmoking volunteers with normal lung function were invited

| Clinical Characterization of Patients With Pulmonary Arterial Hypertension |
|-------------------------------------------------|-----------------|-----------------|-----------------|
| Control (n=5) | IPAH (Fuwai Hospital, Beijing) (n=8) | IPAH (Hammersmith Hospital, London) (n=10) | Connective Tissue Disease (n=2) |
| Female sex, % | 20.0 | 87.5 | 70 | 100 |
| Age, y | 45±21 | 36±18 | 44±12 | 42±10 |
| Functional class, n (%) | | | | |
| I | … | 0 | 0 | 0 |
| II | … | 3 (37.5) | 7 (70) | 0 |
| III | … | 5 (62.5) | 3 (30) | 2 (100) |
| IV | … | 0 | 0 | 0 |
| Mean arterial pressure, mm Hg | 84±7 | 88±12 | 85±7 | 98±2 |
| Cardiac output, L/min | … | 4.0±1.4 | 4.2±1.2 | 4.7±0.2 |
| Cardiac index, L/min per m² | … | 2.7±0.8 | 2.3±0.6 | 2.8±0.1 |
| Right atrial pressure, mm Hg | … | 5.4±5.1 | 8.9±3.7 | 6.0±1.4 |
| mPAP, mm Hg | … | 52.6±12.8 | 53.2±12.1 | 55.5±29.0 |
| PAP sys, mm Hg | … | 81.5±19.1 | 81.2±20.4 | 91.0±41.0 |
| PAP dIA, mm Hg | … | 36.7±7.7 | 31.5±9.1 | 35.5±20.5 |
| TPR, dyne·s·cm⁻⁵ | … | 1021±345 | 1190±608 | 1097±696 |
| PVR, dyne·s·cm⁻⁵ | … | 792±312 | 793±484 | 784±619 |
| 6MWD, m | … | 365±75 | 433±109 | 326±279 |
| FDG uptake, score | 2.02±0.71 | 3.38±1.26* | 3.17±1.23* | 6.80±0.89 |

**Table 1.** Clinical Characterization of Patients With Pulmonary Arterial Hypertension

FDG indicates 2-fluoro-2-deoxyglucose; IPAH, idiopathic pulmonary arterial hypertension; mPAP, mean pulmonary artery pressure; 6MWD, 6-minute walk distance; PAP sys, systolic pulmonary artery pressure; PAP dIA, diastolic pulmonary artery pressure; PVR, pulmonary vascular resistance; and TPR, total pulmonary vascular resistance.

*P<0.05 compared with control group.
to participate. Patients with diabetes mellitus or glucose intolerance were excluded. The project was approved by the local research ethics committees of the respected institutions (Hammersmith Hospital reference No. 09/H0706/53 and Fuwai Hospital project No. 216), and all patients gave written informed consent.

All patients (Table 1) underwent right heart catheterization within 1 week of PET imaging. Right atrial and right ventricular (RV), pulmonary arterial (PAP) and pulmonary capillary wedge (PCWP) pressures were recorded. Cardiac output was calculated using the indirect Fick method and was also expressed as the cardiac index to correct for body surface area. Pulmonary vascular resistance was calculated as (mean PAP-PCWP)/cardiac output and total pulmonary resistance as mean PAP/cardiac output. The 6-minute walk test was performed with a standardized protocol.21

Patients recruited from Hammersmith Hospital in London (10 with IPAH) were receiving evidence-based PAH targeted treatments (Table 1). Patients recruited from Fuwai Hospital in Beijing (8 with IPAH and 2 with connective tissue disease) were taking symptomatic relief medications (such as diuretics) at the time of the scan. Intestinal lung disease was excluded in the 2 patients with connective tissue disease by imaging (Figure 1 in the online-only Data Supplement).

Patient FDG PET

After a minimum 8-hour overnight fast, each subject was positioned supine with the thorax in the detector ring of the PET scanner (Truepoint Biography 64, Siemens Healthcare). A cannula was placed in the right arm vein for injection of radioisotope. Transmission scans were performed by measuring the transmission of radioactivity through the field of view of the PET scanner from a ring source containing germanium 68. An initial rectilinear transmission scan was performed to define the diaphragmatic boundary of the lung, and the scanner bed was moved so that the lowest of the 15 transaxial planes measured in the PET scanner was aligned with the dome of the diaphragm. A 20-minute transmission scan was performed for attenuation correction of the subsequent emission scans and for measurement of regional lung density. Dynamic rest imaging was started at the time of FDG intravenous injection (120 MBq), and positron emission data were acquired in 15 consecutive frames over 1 hour (frames 7×180, 7×300, 1×240 seconds).

Data Analysis

Attenuation-corrected data were reconstructed with the use of an iterative algorithm (ordered-subset expectation maximization; 4 iterations, 8 subsets). Ordered-subset images underwent 5-mm full-width-at-half-maximum gaussian postsmoothing. The matrix was 256×256. Attenuation-corrected data were reconstructed with the use of an iterative algorithm (ordered-subset expectation maximization; 4 iterations, 8 subsets). Ordered-subset images underwent 5-mm full-width-at-half-maximum gaussian postsmoothing. The matrix was 256×256.

The region of interest was finalized with 1-cm automated erosion from the top to the bottom of the LV and adjusted by PET thresholding. The input function and the whole-lung TAC were used to estimate the influx rate constant K of the 2-tissue irreversible compartment model according to the modified Patlak plot, with t* set to 20 minutes. The FDG uptake score was also computed on a per-pixel basis, typically 200 000 voxels (2 mm² per voxel) for a human subject, and a parametric 3-dimensional map was generated accordingly. A 2-tissue irreversible compartment model (Figure II in the online-only Data Supplement) was also fitted to the whole-lung TAC with the image-derived blood TAC as input function to estimate the rate constant k (rate of phosphorylation). The 2-tissue irreversible model was used because it always fitted the data better than a 2-tissue reversible model according to the standard Akiya information criterion in its small sample formulation.22

Animals and Experimental Design

Adult male Sprague-Dawley rats (weight, 200–250 g; from Charles River, UK) were used. All experiments were conducted in accordance with the UK Home Office Animals (Scientific Procedures) Act 1986 (London, UK). Pulmonary hypertension was induced by subcutaneous injection of monocrotaline (60 mg/kg; Sigma-Aldrich). The following studies were performed:

18FDG PET: After Monocrotaline-Induced Pulmonary Hypertension Progression

18FDG PET scans were performed in control and monocrotaline rats 1, 2, and 4 weeks after the monocrotaline injection (n=4–6 per group).

18FDG PET: To Assess the Effects of Treatment

Rats were divided into 5 groups (n=6 per group): (1) a control group; (2) a group exposed to monocrotaline for 4 weeks (monocrotaline group); (3) a group exposed to monocrotaline followed by dichloremacetate (70 mg/kg per day, drinking water; Sigma-Aldrich); (4) a group exposed to monocrotaline followed by imatinib (100 mg/kg per day, oral gavage; LC Laboratories); and (5) a group exposed to monocrotaline followed by sunitinib (5 mg/kg BID, oral gavage; Pfizer). Each drug treatment was started 2 weeks after monocrotaline injection and continued for an additional 2 weeks. At the end of each treatment, 18FDG PET scans were performed, and tissues were collected for biochemical and histological examination.

18FDG PET: Longitudinal Assessment for Early Treatment Responder

Three weeks after monocrotaline injection, rats were treated with dichloremacetate (70 mg/kg per day) or imatinib (100 mg/kg per day) for 7 days. Consecutive 18FDG PET scans were performed before the treatments started and 2 and 7 days after the treatment.

In Vivo 18FDG PET

In vivo imaging was performed with the use of a Siemens Inveon small-animal multimodality PET/CT system (Siemens Healthcare Molecular Imaging). Briefly, after fasting overnight,22 rats were anesthetized with isoflurane (2% to 4%) and positioned in a thermoregulated ventilator (Hammersmith Hospital Respiratory and ECG3 were monitored. Attenuation correction was achieved via a 3–bed position CT scan with 80 kV (peak) and 500 µA, with 220 projections. After the completion of the CT scan, 18FDG (∼40 MBq, <0.5 mL) was injected through a tail vein catheter. Dynamic emission scans were acquired in list mode for 60 minutes with the use of conventional full-ring, whole-body PET. During PET scanning, serial blood samples (20 µL each) were taken via a femoral artery line, with 8 samples at the first minute and then at 2, 3, 5, 10, 15, 30, 45, and 60 minutes, from which plasma was obtained and radioactivity measured to provide a sampled arterial plasma TAC.

At the end, animals were euthanized, and tissue samples (lung, RV/LV, kidney, liver) were collected for measuring radioactivity in a gamma counter and snap-frozen for biochemical measurement. The ratio of RV to LV plus the septum mass (RV/LV+septum) was used as an index of RV hypertrophy. Left lungs were inflated and fixed with 4% formalin for histology examination. The tissue activity was corrected for radioactivity decay and expressed as percentage of injected dose per gram of tissue.

Data Analysis

PET image data were sorted into 0.5-mm sinogram bins and 33 time frames for image reconstruction with the use of filtered back projection with CT-based attenuation correction. The frame durations used for the study were 12×5, 4×15, 6×30, and 11×300 seconds. The reconstructed PET/CT images were analyzed with the use of Inveon Research Workplace software (Siemens Healthcare Molecular Imaging). The whole-lung tissue TAC was calculated from lung PET images coregistered with region of interest on CT lung images, covering the lung volume with clearly visible boundaries adjusted by CT.

Animals and Experimental Design

Adult male Sprague-Dawley rats (weight, 200–250 g; from Charles River, UK) were used. All experiments were conducted in accordance with the UK Home Office Animals (Scientific Procedures) Act 1986 (London, UK). Pulmonary hypertension was induced by subcutaneous injection of monocrotaline (60 mg/kg; Sigma-Aldrich). The following studies were performed:

18FDG PET: After Monocrotaline-Induced Pulmonary Hypertension Progression

18FDG PET scans were performed in control and monocrotaline rats 1, 2, and 4 weeks after the monocrotaline injection (n=4–6 per group).

18FDG PET: To Assess the Effects of Treatment

Rats were divided into 5 groups (n=6 per group): (1) a control group; (2) a group exposed to monocrotaline for 4 weeks (monocrotaline group); (3) a group exposed to monocrotaline followed by dichloroacetate (70 mg/kg per day, drinking water; Sigma-Aldrich); (4) a group exposed to monocrotaline followed by imatinib (100 mg/kg per day, oral gavage; LC Laboratories); and (5) a group exposed to monocrotaline followed by sunitinib (5 mg/kg BID, oral gavage; Pfizer). Each drug treatment was started 2 weeks after monocrotaline injection and continued for an additional 2 weeks. At the end of each treatment, 18FDG PET scans were performed, and tissues were collected for biochemical and histological examination.

18FDG PET: Longitudinal Assessment for Early Treatment Responder

Three weeks after monocrotaline injection, rats were treated with dichloroacetate (70 mg/kg per day) or imatinib (100 mg/kg per day) for 7 days. Consecutive 18FDG PET scans were performed before the treatments started and 2 and 7 days after the treatment.

In Vivo 18FDG PET

In vivo imaging was performed with the use of a Siemens Inveon small-animal multimodality PET/CT system (Siemens Healthcare Molecular Imaging). Briefly, after fasting overnight,22 rats were anesthetized with isoflurane (2% to 4%) and positioned in a thermoregulated (37°C) rat bed (M2M). Respiration and ECG3 were monitored. Attenuation correction was achieved via a 3–bed position CT scan with 80 kV (peak) and 500 µA, with 220 projections. After the completion of the CT scan, 18FDG (∼40 MBq, <0.5 mL) was injected through a tail vein catheter. Dynamic emission scans were acquired in list mode for 60 minutes with the use of conventional full-ring, whole-body PET. During PET scanning, serial blood samples (20 µL each) were taken via a femoral artery line, with 8 samples at the first minute and then at 2, 3, 5, 10, 15, 30, 45, and 60 minutes, from which plasma was obtained and radioactivity measured to provide a sampled arterial plasma TAC.

At the end, animals were euthanized, and tissue samples (lung, RV/LV, kidney, liver) were collected for measuring radioactivity in a gamma counter and snap-frozen for biochemical measurement. The ratio of RV to LV plus the septum mass (RV/LV+septum) was used as an index of RV hypertrophy. Left lungs were inflated and fixed with 4% formalin for histology examination. The tissue activity was corrected for radioactivity decay and expressed as percentage of injected dose per gram of tissue.

Data Analysis

PET image data were sorted into 0.5-mm sinogram bins and 33 time frames for image reconstruction with the use of filtered back projection with CT-based attenuation correction. The frame durations used for the study were 12×5, 4×15, 6×30, and 11×300 seconds. The reconstructed PET/CT images were analyzed with the use of Inveon Research Workplace software (Siemens Healthcare Molecular Imaging). The whole-lung tissue TAC was calculated from lung PET images coregistered with region of interest on CT lung images, covering the lung volume with clearly visible boundaries adjusted by CT.
density thresholding. Cumulative images over 0 to 60 minutes were used for kinetic analysis of tracer uptake. The arterial input function was determined individually by sampled arterial plasma TAC or the PET image–derived activity curve from the LV blood pool. Kinetic influx rate constant \( K_i \) was estimated from the slope of the linear regression on the Patlak plot and normalized with the volume of distribution (the intercept of the Patlak linear regression). The rate of glycolysis was calculated (\( \frac{[\text{glucose}]}{K_i} \)). Spatial distribution of FDG uptake within the lungs was mapped from parametric images transformed from voxel-wise \( K_i \) values,\(^{26,27}\) and a histogram was generated accordingly.

**Histology Examination**

Transverse rat lung sections were processed for elastic van Gieson and hematoxylin and eosin staining. Vessels <100 \( \mu \)m in peripheral lung were counted under a microscope (×40), and pulmonary vascular remodeling was expressed as the proportion of vessels with double elastic lamina to total vessels counted. Lung sections were also processed for Ki-67 (1:50; Thermo Scientific), CD68 (1:50; Serotec), and GLUT1 (1:50; Abcam) immunostaining. Fifty peripheral vessels (<100 \( \mu \)m) were selected per slide, and the number of Ki-67 per vessel was scored. CD68-positive cells were counted in whole-lung tissue sections and expressed as per square millimeter. Double immunofluorescence with CD68 and GLUT1 was performed with the use of fluorescence secondary antibodies anti-mouse Alexa 488 and anti-rabbit Alexa 568 (1:2000; Invitrogen). Images (green for GLUT1 and red for CD68) were detected under a Leica confocal microscope (TCS SP2 AOBs).

**Western Blotting**

Rat lung samples were homogenized in RIPA buffer (150 mmol/L sodium chloride, 1.0% NP-40 or Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mmol/L Tris, pH 8.0, supplemented with protease inhibitor; Roche). Western blotting was performed according to the manufacturer’s suggestions (rabbit polyclonal anti-GLUT1 1:1000; Abcam). Proteins were detected by Novex ECL chemiluminescence (Invitrogen). Optical densities of individual bands were measured, and protein expression was standardized with \( \beta \)-actin.

**Cell Culture**

**Proliferation Assay**

Pulmonary artery adventitial fibroblasts isolated from the lungs of IPAH patients and healthy donors were obtained from the University of Giessen and Marburg Lung Center tissue bank (Table I in the online-only Data Supplement). To measure proliferation, cells (passages 4–6) were cultured on poly-lysine (ScienCell)–coated dishes in fibroblast medium (ScienCell) to 90% confluence in 96 well plates, washed, and serum starved for 24 hours. Growth medium was added at 37°C. After removal of the medium, the cells were washed with PBS and lysed in 300 \( \mu \)L of 0.2 mol/L NaOH. Cell-bound radioactivity was measured in 150-\( \mu \)L aliquots of lysate by a well gamma counter, and the rest was used for protein quantification analysis (Modified Lowry Protein Assay Reagent; Pierce Chemical Co). Cellular \(^{18}\)FDG uptake was defined as uptake/mg protein (%)=cell-bound radioactivity per wells/added radioactivity/protein (mg) per wells.

**Statistical Analysis**

Data are presented as mean±SEM. Normal distribution was verified with the Kolmogorov-Smirnov test, and variance of homogeneity was tested by the Levene test. Differences between groups were assessed by either the Student t test or an appropriate ANOVA followed by the Bonferroni post hoc test for multigroup comparisons. Correlations between FDG uptake (score and \( k_i \)) and clinical variables were determined by Pearson linear regression. A \( P \) value <0.05 was considered statistically significant. All statistical analyses were performed with the use of Prism 5.0 (GraphPad Software, La Jolla, CA).

**Results**

**Increased Lung \(^{18}\)FDG Uptake in IPAH Patients**

Sixty-minute dynamic data acquisition enabled calculation of the rate of lung \(^{18}\)FDG uptake per unit of lung volume\(^{22,23}\) and measurement of the rate of glucose phosphorylation (\( k_i \)) with the use of a 2-tissue compartment model (Figure II in the online-only Data Supplement).\(^{23,30}\)

Overall, lung \(^{18}\)FDG uptake in the IPAH patient group (FDG score: 3.27±1.22) was increased compared with controls (2.02±0.71; \( P<0.05 \)) (Figure I and Figure III in the online-only Data Supplement). Two patients with PAH associated with connective tissue disease showed the highest \(^{18}\)FDG uptake (5.07 and 7.11). However, there was variation within the IPAH population, such that some patients exhibited uptake 3-fold above the control group, whereas others were in the range of controls. Two-tissue compartment model analysis demonstrated a significantly higher \( k_i \) (Figure 1B) in IPAH lungs (0.016±0.005) than in control subjects (0.01±0.002; \( P<0.05 \)), consistent with increased intracellular glucose metabolism in IPAH patients, but again with a range of values.

Further analysis of IPAH patients with a high \(^{18}\)FDG uptake revealed an uneven distribution of \(^{18}\)FDG uptake within the lungs (Figure 1C). Three-dimensional parametric mapping of computed per-voxel FDG scores from IPAH patients demonstrated that focal areas of relatively high uptake were distributed throughout the lung parenchyma (Figure 1D) with no consistent pattern.
Lung \(^{18}\text{FDG}\) uptake (FDG score and \(k_3\)) did not correlate with 6-minute walking distance, mean pulmonary artery pressure, or pulmonary vascular resistance in the IPAH group (Figure IV in the online-only Data Supplement).

Increased Lung \(^{18}\text{FDG}\) Uptake in a Monocrotaline Model

To understand the relationship between lung \(^{18}\text{FDG}\) uptake and vascular pathology, studies were conducted in a rodent model of pulmonary hypertension. Rapid \(^{18}\text{FDG}\) uptake was detected in the heart and liver of monocrotaline rats, whereas \(^{18}\text{FDG}\) accumulation in the lung was slower. Static images obtained during the last phase of dynamic PET acquisitions revealed significantly higher accumulation of \(^{18}\text{FDG}\) in the monocrotaline rat lung (Figure 2A). Dynamic 60-minute PET data acquisition was used for kinetic analysis. Figure 2B represents an example of the plasma activity curve plotted against the image-derived activity curve from the LV blood pool showing comparable arterial input function calculations from both methods. A representative Patlak plot is shown in Figure 2C.

Histological examination (Figure 3A) of the monocrotaline lung confirmed progressive vascular remodeling over 1, 2, and 4 weeks after monocrotaline injection. The remodeled vessels demonstrated Ki-67 and GLUT1 expression (Figure 3A and Figure V in the online-only Data Supplement) and prominent perivascular infiltration of macrophages (CD68) (Figure 3A). Prominent GLUT1 expression was demonstrated in...
perivascular infiltrating macrophages by double immunofluorescence in the monocrotaline rat lung (Figure 3A and 3B).

There was a progressive increase in 18FDG influx (Kᵢ influx rate) (Figure 4A) supported by direct tissue 18FDG measurements (Figure 4B) in the monocrotaline rat lung in proportion to the vascular pathology (Figure 4C through 4E), Ki-67 staining (R²=0.73, P<0.0001) (Figure 4D), and macrophage accumulation (Figure 4E). Calculated glycolysis rates ([glucose×Kᵢ]) were increased significantly in the monocrotaline rat lung, providing evidence of an alteration of cellular bioenergetics relevant to pulmonary pathology.

**Attenuated Lung FDG Uptake With Imatinib, Sunitinib, and Dichloroacetate Treatment in the Monocrotaline Model**

We investigated the response of the 18FDG PET signal to treatments with proven efficacy in rodent models of pulmonary hypertension. Consistent with published data, exposure to dichloroacetate, imatinib, or sunitinib during weeks 2 to 4 after monocrotaline treatment attenuated pulmonary vascular remodeling, as demonstrated by significant reductions in the proportion of remodeled vessels with double elastic lamina (Figure 5A), Ki-67 scores (Figure 5B), inflammatory cell accumulation (Figure 5C and Figure V in the online-only Data Supplement), and reduced RV hypertrophy (RV/LV+septum ratio, 0.48±0.10 for dichloroacetate; 0.32±0.02 for imatinib; 0.33±0.04 for sunitinib; P<0.001 compared with placebo group: 0.60±0.06).

This was accompanied by a significant reduction in FDG influx, Kᵢ, after dichloroacetate, imatinib, and sunitinib treatments (Figure 5D), confirmed by direct measurement of tissue 18FDG uptake (Figure 5E). Glycolysis rates were decreased in all treatment groups compared with the placebo group (Figure 5F). Western blotting demonstrated that lung Glut1 protein expression was decreased by dichloroacetate and imatinib treatments (Figure 5G)

**Decreased FDG Uptake Indicates Early Treatment Response to Imatinib and Dichloroacetate**

To examine the early time course of response, consecutive 18FDG PET scans were performed on days 2 and 7 after dichloroacetate and imatinib treatments were started in the monocrotaline rat. A significant drop in 18FDG uptake was observed 2 days after imatinib treatment but not in the dichloroacetate group (Figure 6A). After 7 days of treatment, lung 18FDG uptake was reduced in both dichloroacetate- and imatinib-treated rats (Figure 6A).

Representative parametric Kᵢ maps of lungs from control and 4-week monocrotaline rats are shown in Figure 6B. The lung Kᵢ histogram plot of the 4-week monocrotaline rat is skewed to the left (Figure 6C). After 2 and 7 days of imatinib treatment, the amplitudes of the Kᵢ histogram were reduced (Figure 6D).

**Imatinib and Dichloroacetate Attenuate Cellular FDG Uptake in IPAH Fibroblasts**

To investigate further the effect of imatinib and dichloroacetate on pulmonary vascular cellular 18FDG uptake, studies were conducted in pulmonary arterial fibroblasts from 3 IPAH patients and 3 donor lungs in culture. Pulmonary arterial fibroblasts from IPAH patients exhibited greater proliferation capacity (Figure 7A) and increased GLUT1, PDK1, and HK1 expression compared with cells from donor lungs; in contrast, PDK4 expression was reduced in IPAH pulmonary arterial fibroblasts (Figure 7B). 18FDG uptake was increased in IPAH pulmonary arterial fibroblasts (155%) compared with donor cells (Figure 7C). Imatinib and dichloroacetate at concentrations that inhibited the growth of pulmonary arterial fibroblasts (Figure 7D and 7E) also reduced cellular 18FDG uptake (Figure 7B) but did not affect uptake in cells from donor lungs (Figure 7B). No cell death was observed with imatinib and dichloroacetate treatments.
We used dynamic 18FDG PET imaging to measure glucose uptake and metabolism in a PAH patient cohort and examine the temporal response to targeted therapy in the monocrotaline rat model of pulmonary hypertension. We found that (1) mean lung 18FDG uptake was increased in IPAH patients compared with healthy controls; (2) there is heterogeneity in lung 18FDG uptake in IPAH both between patients and within the lungs of each patient; and (3) increased lung 18FDG uptake responds within days to drugs that, over weeks, reduce pulmonary vascular remodeling in animal models. Furthermore, detailed kinetic analysis of the lung 18FDG signal revealed increased glucose phosphorylation in IPAH, consistent with increased glucose metabolism in the IPAH lung. 18FDG uptake and gene expression studies in pulmonary arterial fibroblasts isolated from IPAH patients support the concept that a proliferative pulmonary vascular pathology contributes to the lung 18FDG PET signal. Data from the monocrotaline rat model, in which inflammation around small pulmonary artery, is increased in the monocrotaline rat and reduced with dichloroacetate and imatinib treatment. These treatments also reduced the rate of glycolysis.

Interest in the use of 18FDG PET in IPAH is predicated on evidence of abnormal cellular metabolism, notably a glycolytic shift, in proliferating cells, driving the pulmonary vascular changes in human and experimental pulmonary hypertension and referred to as the Warburg effect in cancer biology. 18FDG PET measures the uptake of 18FDG tracer into tissue and can be used to measure both glucose transport into tissue and can be used to measure both glucose transport and phosphorylation in viable cells. Previous studies, reporting standardized uptake values, have shown increased lung parenchymal 18FDG uptake in rats and 4 IPAH patients. Dynamic PET scanning allows more in-depth analysis than static acquisition protocols. In particular, in addition to calculating the rate of tissue glucose uptake (the FDG score, based on the 18FDG influx constant \( K_i \) calculated from Patlak graphic analysis corrected for the initial volume of the distribution), it also permits measurement of the rate of glucose phosphorylation \( k_p \) from a 2-tissue compartment model (Figure II in the online-only Data Supplement). Our data demonstrate not only increased lung FDG uptake but a significantly higher rate of glucose phosphorylation \( k_p \) in a proportion of IPAH patients. These in vivo observations are consistent with the Warburg effect in the pulmonary hypertensive lung.
It is notable that the $^{18}$FDG signal from IPAH lungs is not homogeneous. The heterogeneity is evident between patients from the distribution of measurements of both $^{18}$FDG influx and $K_i$. Some IPAH patients exhibited uptake 3-fold above that of the control group, whereas others were in the range of controls. It is also evident within the lungs of an IPAH patient with an increased $^{18}$FDG signal. Three-dimensional parametric mapping of voxel-wise $^{18}$FDG scores revealed a nonuniform distribution of $^{18}$FDG uptake. The observation of intervariability/intravariability in lung $^{18}$FDG measurements in IPAH patients aligns with our current understanding of the disease in terms of both the presentation and clinical course of the disease as well as after recent reports of extensive morphological heterogeneity within individual lungs and between lungs from IPAH patients.\(^{3,33}\)

Studies in the monocrotaline model demonstrated a close correlation between lung $^{18}$FDG uptake and pulmonary vascular remodeling as measured by morphological changes and proliferative and inflammatory markers, as reported previously.\(^{9}\) Furthermore, treatment with dichloroacetate, imatinib, and sunitinib attenuated pulmonary hypertension and vascular remodeling in this model and reduced Glut1 expression, and the $^{18}$FDG PET lung signal was reduced in association with decreased peripheral vascular muscularization and inflammatory cell accumulation. Direct correlation of $^{18}$FDG uptake with vascular remodeling in humans is not possible. With this limitation, studies were conducted on pulmonary fibroblasts isolated from IPAH patients as an in vitro model system. The use of the fibroblast as a model cell to explore the biology and pharmacology in vitro was based on the well-supported view that the fibroblast is an important contributor to the vascular pathology of PAH.\(^{34,35}\) Consistent with this, pulmonary artery fibroblasts from IPAH patients exhibited a hyperproliferative phenotype and increased expression of Glut1, PDK1, and HK1, compatible with increased glycolytic metabolism. $^{18}$FDG uptake was increased in IPAH fibroblasts compared with cells from control lobectomy specimens. Both the metabolic modulator dichloroacetate and the tyrosine kinase inhibitor imatinib reduced $^{18}$FDG uptake in IPAH pulmonary fibroblasts along with their antiproliferative effect.

It is recognized that in addition to the fibroblast, the obliterative pulmonary remodeling in IPAH involves other cellular elements of the vessel wall, including endothelial cells and smooth muscle cells,\(^{3,33}\) as well as perivascular infiltration of inflammatory cells.\(^{3,36}\) $^{18}$FDG PET imaging does not discriminate between these cell types. Indeed, Xu et al\(^{16}\) have shown that pulmonary arterial endothelial cells isolated from IPAH patients have a higher glycolytic rate than control cells. Inflammatory cells, such as activated macrophages, also use exogenous glucose as a fuel and show increased expression of Glut1 and Glut3 as well as hexokinase.\(^{37,38}\) We demonstrated prominent GLUT1 expression in perivascular macrophages in monocrotaline rat lung. In addition, $^{18}$FDG uptake was most pronounced in 2 patients with PAH associated with connective tissue lung disease. Inflammation is thought to play a particularly prominent role in the pathology of this form of PAH. At present, we must assume that $^{18}$FDG PET reports glucose metabolism from both the hyperproliferative and inflammatory cellular components of the vascular pathology in PAH.

$^{18}$FDG PET lacks specificity as a clinical tool for the diagnosis of PAH. Furthermore, lung $^{18}$FDG uptake did not correlate with any of the clinical markers of IPAH severity. This is not surprising. Little is known about the natural history of IPAH and the temporal relationship between the proliferative and inflammatory processes that underlie the disease and the change in pulmonary vascular resistance. Nonetheless, $^{18}$FDG PET may be useful as a tool to stratify patients with IPAH for particular therapies. There is a clear precedent in oncology.
In recent years, 18FDG PET has been widely used for monitoring tumor response to therapy, including imatinib. The metabolic response reported by 18FDG PET in oncology clearly precedes an anatomic response and is predictive of outcome. In this respect, the early reduction of 18FDG uptake in the rat monocrotaline model within 2 days of initiating imatinib treatment and 7 days of starting dichloroacetate is of considerable interest. A rapid response to both treatments was also observed in vitro. Exposure to dichloroacetate (50 mmol/L) and imatinib (25 µmol/L) reduced 18FDG uptake in IPAH pulmonary fibroblasts in culture within 20 hours. This is consistent with the pharmacology of these agents. Dichloroacetate inhibits pyruvate dehydrogenase kinase and increases the flux of pyruvate into mitochondria, promoting glucose oxidation over glycolysis. Imatinib, an inhibitor of the kinase BCR-ABL and platelet-derived growth factor receptor, decreases glucose uptake by translocation of GLUT1 transporters from the cell membrane into the cytosol and by inhibiting glycolysis and promoting mitochondrial oxidative glucose utilization. Although speculative, it is possible that 18FDG PET may be helpful in early identification of those patients with IPAH with high lung 18FDG uptake who might benefit from, for example, imatinib, by showing an early reduction in 18FDG signal on treatment while sparing those patients who might not benefit but are at risk of side effects.

In conclusion, dynamic 18FDG PET imaging identifies a subpopulation of IPAH patients with increased lung glucose metabolism. 18FDG PET merits further evaluation as a clinical tool to deep phenotype patients with IPAH in studies exploring efficacy and dose-response relationships of new PAH-targeted treatments.

Sources of Funding
This research is supported by project grants from the British Heart Foundation (PG/10/034/28338), Medical Research Council (G0701637/85257), and Pfizer Ltd. We thank the National Institute for Health Research/Wellcome Trust Imperial Clinical Research Facility for supporting the clinical studies.

Disclosures
None.

References
1. Hassoun PM, Mouthon L, Barbera JA, Eddahibi S, Flores SC, Grimminger F, Jones PL, Maitland ML, Michelakis ED, Morrell NW, Newman JH,
Heterogeneity in Lung 18FDG Uptake in PAH

Zhao et al

1223


**CLINICAL PERSPECTIVE**

Pulmonary arterial hypertension (PAH) is a disease of progressive vascular remodeling, characterized by dysregulated growth of pulmonary vascular cells and inflammation. This study explores the application of molecular imaging with the use of positron emission tomography as a tool to understand the vascular pathology of PAH and as a bridging biomarker to address the challenge of assessing therapies directed at structural remodeling in PAH. It examines and utilizes the “Warburg” concept that proliferating cells require more glucose. With application of dynamic fluorine-18–labeled 2-fluoro-2-deoxyglucose (18FDG) positron emission tomography data acquisition and kinetic modeling, our data reveal interindividual and intraindividual variability in 18FDG uptake in the lungs of patients with idiopathic PAH. Using an animal model and vascular endothelial cells from idiopathic PAH lungs, we report that the new treatments under development for PAH inhibit glucose metabolism as well as proliferation. We suggest that 18FDG positron emission tomography may offer a tool for deep phenotyping and stratifying idiopathic PAH patients to better understand the response to new PAH-targeted treatments in a clinical trial.
Heterogeneity in Lung $^{18}$FDG Uptake in Pulmonary Arterial Hypertension: Potential of Dynamic $^{18}$FDG Positron Emission Tomography With Kinetic Analysis as a Bridging Biomarker for Pulmonary Vascular Remodeling Targeted Treatments


*Circulation*. 2013;128:1214-1224; originally published online July 30, 2013; doi: 10.1161/CIRCULATIONAHA.113.004136

*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/128/11/1214

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2013/07/30/CIRCULATIONAHA.113.004136.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation* 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 *Circulation* is online at:
http://circ.ahajournals.org//subscriptions/
Supplementary table 1. The characteristics of 3 individual IPAH patients and 3 individual controls that we used for deriving pulmonary fibroblasts.

<table>
<thead>
<tr>
<th>Type</th>
<th>Age</th>
<th>Sex</th>
<th>PAP (mmHg)</th>
<th>SBP (mmHg)</th>
<th>NYHA class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor</td>
<td>63</td>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor</td>
<td>61</td>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor</td>
<td>42</td>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPAH</td>
<td>16</td>
<td>Male</td>
<td>75</td>
<td>110</td>
<td>IV</td>
</tr>
<tr>
<td>IPAH</td>
<td>50</td>
<td>Female</td>
<td>66</td>
<td>88</td>
<td>III</td>
</tr>
<tr>
<td>IPAH</td>
<td>16</td>
<td>Female</td>
<td>98</td>
<td>140</td>
<td>IV</td>
</tr>
</tbody>
</table>

Supplementary figure 1. There are no signs of interstitial fibrosis in the lungs from the two patients with collagen vascular disease, according to (A) CT or (B) X-ray.
Supplementary figure 2. Compartmental modelling of pulmonary $^{18}$FDG kinetics separates the $^{18}$FDG signal into free and bound tracer and quantifies three parameters: forward tracer diffusion ($K_1$), backward tracer diffusion ($K_2$), and rate of $^{18}$FDG phosphorylation, i.e. glucose metabolism ($K_3$). The amount of phosphorylated FDG is directly related to cellular glucose metabolic activity.

Supplementary figure 3. Lung $^{18}$FDG uptake in IPAH patients recruited from both Beijing Fuwai hospital (FW) (3.48 ± 1.20) and London Hammersmith hospital (HH) (3.27 ± 1.12) was increased compared to controls (2.02±0.71, $P<0.05$)

Supplementary figure 4. Lung $^{18}$FDG uptake (FDG score) did not correlate with A. six-minute walking distance (6MWD), B. mean pulmonary artery pressure (mPAP) or C. pulmonary vascular resistance (TPR) in the IPAH group.
Supplementary figure 5. A. Immunofluorescence with GLUT1 (green, left) and double immunofluorescence with GLUT1 (green) and CD68 (red) (right) in MCT lung sections. GLUT1 expression is observed in the remodelled vessel (red arrow) and perivascular infiltrating macrophages. B. Representative pictures of immunostaining with GLUT1 in lung sections of control, MCT, and MCT treated with DCA or imatinib (IMA). We observed reduced GLUT1 expression in lung sections with DCA and imatinib treatment.