Severe pulmonary hypertension is associated with altered right ventricle metabolic substrate uptake

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Graham BB, Kumar R, Mickael C, Sanders L, Gebreab L, Huber KM, Perez M, Smith-Jones P, Serkova NJ, Tuder RM. Severe pulmonary hypertension is associated with altered right ventricle metabolic substrate uptake. Am J Physiol Lung Cell Mol Physiol 309: L435–L440, 2015. First published June 26, 2015; doi:10.1152/ajplung.00169.2015. — In severe pulmonary hypertension (SPH), prior studies have shown an increase in right ventricle (RV) uptake of glucose, but it is unclear whether there is a change in the relative utilization of fatty acids. We hypothesized that in the RV in SPH, as in left ventricular (LV) failure, there is altered substrate utilization, with increased glucose uptake and decreased fatty acid uptake. SPH was induced in rats by treatment with the VEGF receptor inhibitor SU5416 and 3 wk of hypoxia (10% FIO2), followed by an additional 4 wk of normoxia (SU-Hx group). Control rats were treated with carboxymethylcellulose vehicle and 7 wk of normoxia (CMC-Nx group). The rodents then underwent positron emission tomography with sequential administration of two radiotracers, 2-deoxy-2-[18F]fluoro-glucose (18F-FDG) and 14-(R,S)-[18F]fluoro-6-thia-heptadecanoic acid (18F-FTHA), analogs of glucose and fatty acid, respectively. We used female Sprague-Dawley rats (Charles River, 4–6 wk old at the start of the experiment. As previously described (1, 19), we administered a subcutaneous injection of 20 mg/kg of SU5416 (SU; Cayman) dissolved in carboxymethylcellulose (CMC) and mixed with PBS, or the equivalent volume of CMC/PBS alone (Fig. 1A). SU-treated rats were placed in 10% FiO2 hypoxia chamber for 3-wk duration; CMC-treated rats were maintained in normoxia (Nx) (at Denver altitude: 1,560 m). Then both groups were maintained in normoxia for 4 additional wk. At the time of imaging, the rats weighed an average of 253 g.

METHODS

Rat model of SPH. We used female Sprague-Dawley rats (Charles River). Animals were fasted for 4 h prior to imaging. The rodents were initially sedated with inhaled isoflurane (2.5%), the tail vein was cannulated, and the anesthesia was then switched to intravenous propofol (0.7 mg·kg⁻¹·min⁻¹). The animals remained anesthetized throughout the imaging protocol. The rodent was placed onto a temperature-controlled animal bed and inserted into an Inveon micro-

metabolic phenotype in which glucose is the predominant energy source (3, 17).

It has been hypothesized that a shift in metabolic substrate may occur in the RV as well. Several studies have shown increased uptake of the glucose analog 2-deoxy-2-[18F]fluoro-glucose (18F-FDG) in the RV in pulmonary arterial hypertension (PAH) patients (2, 9, 12, 22). 18F-FDG uptake in the RV is also increased in the monocrotaline rat model of pulmonary hypertension (PH) (13, 18).

It is unclear whether there is a change in fatty acid uptake and metabolism in the failing RV in SPH. We hypothesized that, in the RV in SPH, there is a shift in substrate utilization similar to that seen in end-stage LV failure, with increased glucose uptake and decreased fatty acid uptake. We used a model of SPH induced by combined VEGF inhibition and hypoxia, followed by additional normoxia to exacerbate the phenotype (1, 19). We performed sequential imaging using 18F-FDG and 14-(R,S)-[18F]fluoro-6-thia-heptadecanoic acid (18F-FTHA), glucose and fatty acid analogs, respectively. We observed that in this model of SPH there was a significant decrease in 18F-FTHA uptake and a mild increase in 18F-FDG uptake in the RV, suggestive of a major shift in RV substrate preference.

THE INCREASED RIGHT VENTRICULAR (RV) afterload present in severe pulmonary hypertension (SPH) results in progressive RV failure, the major cause of death in this disease. In the healthy myocardium, 95% of ATP is produced from oxidative phosphorylation, with 60–90% of the substrate from β-oxidation of fatty acids and the rest from glucose metabolism (16, 17). In compensated or early left ventricular (LV) failure there appears to be an increase in fatty acid metabolism, which transitions to decreased fatty acid metabolism in decompensated or late LV failure (3, 17, 20, 23). There may be a concurrent increase in glucose metabolism, suggestive of a shift in substrate utilization and potentially reversion to a fetal pulmonary hypertension; right ventricle; metabolism; fatty acid oxidation

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A PET scanner (Siemens, Knoxville, TN). 18F-FDG (average of 474.2 MBq) or 18F-FTHA (average of 447.4 MBq) was administered via syringe pump into the tail vein cannula in a total volume less than 120 µl. Images were taken immediately prior to, during, and up to 40 min after radiotracer injection. The PET data were analyzed by using the following dynamic frames: 10 min, 4 × 5 min, and 1 × 10 min. PET images were reconstructed by an OSEM+MAP algorithm, and data analysis was performed with an Inveon Research Workstation (v1.4.3, Siemens). Results are presented as Standardized Uptake Values (corrected for dose, body weight, and radioactive decay), normalized to the control group RV signal.

RT-PCR assessment of the fatty acid metabolism pathway. RNA was isolated from RV tissue by use of the Qiagen miRNAeasy mini kit. RNA integrity was confirmed (Agilent Bioanalyzer), with all samples having RNA integrity > 9.0. cDNA was synthesized from 0.5 µg of RNA (Bio-Rad iScript cDNA kit). Gene expression was determined by using the Qiagen RT2 Profiler PCR Array for rat fatty acid metabolism (PARN-007ZA-6) and cycled by use of an Applied Biosystems 7500 Real-Time PCR System.
Cardiac Substrate Uptake in PH

Results

Western blot and immunohistochemistry. A sample of frozen RV tissue was disrupted and sonicated in PBS containing antiproteases, and protein concentration was determined by Bradford assay (Bio-Rad). We measured 50 μg of protein from each sample by Western blot with mouse anti-lipoprotein lipase (Abcam ab93898, 1:1,000 dilution) and β-actin (Cell Signaling no. 4967, 1:20,000 dilution), horseradish peroxidase anti-mouse (Vector PI2000, 1:5,000 dilution), and detection by ECL (GE Healthcare RPN2106).

Formalin-fixed and paraffin-embedded sections were immuno- stained for lipoprotein lipase. Slides were deparaffinized and antigen retrieved by boiling for 20 min in citrate buffer (Vector H-3300). The slides were rinsed in Tris-buffered saline (TBS) with 0.05% Tween 20 (TBST), and 10% horse serum in TBS applied for 60 min. The block was removed and the primary antibody (Abcam ab93898, 1:400 dilution in TBS) applied overnight at 4°C. The slides were rinsed in TBST and 1:200 AF488 donkey anti-mouse (Invitrogen A21202) was applied for 60 min. The slides were rinsed and Vectashield with DAPI (Vector H-1500) was applied for coverslipping. For vascular colocalization, TRITC-conjugated lectin from Bandeiraea simplicifolia was added to the primary antibody mixture (1:50 dilution; Sigma L5264). Images were acquired on a Nikon Eclipse E800 microscope with ×40 or ×60 oil objectives via a black-and-white charge-coupled device camera (Photometrics, Tucson, AZ), with Nikon NIS Elements Software v3.2.

Statistics. Statistical analysis was performed in Microsoft Excel. Student’s t-test was used to compare the results between two groups. P < 0.05 was considered statistically significant.

18F-FDG uptake. The rats first underwent 18F-FDG imaging. We observed that, compared with CMC-Nx rats, the uptake of 18F-FTHA in SU-Hx rats appeared to decrease in both the RV and LV free walls (Fig. 1C). Quantification (Fig. 2B) showed there was a significant decrease in 18F-FTHA in the RV tissue in SU-Hx rats relative to CMC-Nx rats (average RV/LV = 0.64; SU-Hx average RV/LV = 0.69; for both groups P = not significant).

Ratio of 18F-FDG to 18F-FTHA. As a measure of relative substrate utilization in the myocardium, we compared 18F-FDG to 18F-FTHA uptake in the same animals (Fig. 2C). Relative to CMC-Nx rats, the RV of SU-Hx rats had a significant increase in the ratio of 18F-FDG to 18F-FTHA uptake (average RV 18F-FDG/18F-FTHA ratio of 6.0 for SU-Hx rats vs. 2.0 for CMC-Nx rats; P < 0.05), indicating a shift insubstrate utilization toward decreased fatty acid and increased glucose uptake in the RV. There was no change in the in the ratio of 18F-FDG uptake to 18F-FTHA uptake in the LV between the two groups (average LV 18F-FDG/18F-FTHA ratio of 3.0 in both groups; P = not significant), indicating no shift in relative LV substrate utilization.

Assessment of fatty acid metabolism by RT-PCR. To gain insight into possible mechanisms by which fatty acid metabolism may be suppressed in SU-Hx RV tissue, RNA was isolated and quantified for transporters and enzymes in the fatty acid metabolism by use of an RT-PCR array. We found significant mRNA downregulation at multiple steps in the fatty acid metabolism pathway in SU-Hx RV tissue (Fig. 3: ratios of gene expression expressed as means ± 95% confidence interval). This includes enzymes that break down fatty acids in the...
circulation (lipoprotein lipase), fatty acid transporters into the cytoplasm (Slc27a1), fatty acid transporters into the mitochondria (CPT2), β-oxidation enzymes (Acaa2, otherwise known as 3-ketoacyltransferase), and fatty acid synthesis proteins (Gk2).

Confirmatory assessment of lipoprotein lipase. To corroborate that fatty acid transport into SU-Hx RV tissue was decreased, we assessed protein levels of lipoprotein lipase in RV tissue. We selected lipoprotein lipase because this is the first step in fatty acid metabolism and a potentially a key regulatory step. By Western blot the concentration of lipoprotein lipase protein in the RV trended toward decrease (Fig. 4, A and B). We also observed that SU-Hx rats had less lipoprotein lipase than CMC-Nx rats by immunostaining (Fig. 4C). Of note, lipoprotein lipase expression localized to capillaries, as identified by lectin costaining (Fig. 4D).

DISCUSSION

We found that rats with SPH had a shift in RV metabolic substrate uptake, with a significant decrease in fatty acid analog uptake and a mild increase in glucose analog uptake. The decrease in fatty acid uptake suggested by the metabolic imaging studies was corroborated by a global decrease in fatty acid transporters and enzymes in the RV tissue (at the mRNA level) and a specific decrease in lipoprotein lipase at the protein level.

We used 18F-FTHA as the fatty acid analog radiotracer for this study. The thia substitution in FTHA (sulfur replaces the 6th carbon) blocks fatty acid β-oxidation, avoiding radioactive metabolite redistribution. Other fatty acid analogs have also been used as radiotracers in studies of myocardial metabolism, including 18F-fluoro-4-thia-palmitate and 18F-methyl-2-[123I]iodophenyl-pentadecanoic acid (BMIPP). BMIPP is limited by complex kinetics that may not correlate well to fatty acid oxidation in heart failure (15).

Limitations of this study include small numbers (particularly in the SU-Hx group). The experiment was initiated with larger numbers, but many animals died at the time of PET imaging, likely because of the combination of sedation and intravenous administration of the volume of radiotracer. Another limitation is the use of propofol as a sedative during PET imaging; propofol can decrease mitochondrial function (21), which could theoretically decrease fatty acid oxidation and increase glycolysis, and the lipid vehicle may result in decreased fatty acid uptake as well. There may also have been a change in the metabolism phenotype in the 1 wk delay between 18F-FDG and 18F-FTHA, although some delay is required to avoid overlapping radiotracer signal.

In human PAH, multiple studies have shown an increase in RV uptake of 18F-FDG (6–10). 18F-FDG uptake has also been reported to be increased in the monocrotaline rat model of PH, although the magnitude of this increase lessens as the disease becomes decompensated (13, 18). The mild increase we observed in SU-Hx rats with SPH is consistent in this regard. The relative decrease in glucose uptake as the PH becomes more severe could be due to decreased substrate delivery in a low-cardiac-output state, or to decreased glucose transporter or glycolysis enzyme activity. We also observed a decrease in LV 18F-FDG uptake in SPH, which could similarly be due to decreased substrate delivery or decreased tissue-level utilization.

The studies assessing cardiac fatty acid metabolism in human PAH have used BMIPP imaging. In a series of 46 patients with PH, the ratio of RV to LV BMIPP uptake correlated positively with mean pulmonary artery (PA) pressure (10). Of note, these patients had relatively mild pulmonary vascular disease, with 41 having a mean PA pressure less than 30 mmHg. A second study of 21 PH patients used both BMIPP and 99mTc-2-methoxyisobutylisonitrile (MIBI), the latter a marker of myocardium perfusion (11). The authors observed a positive correlation between mean PA pressure and both MIBI and BMIPP uptake. However, 8 patients had lower BMIPP than MIBI uptake: these 8 patients had lower RV ejection fraction and higher mortality than the patients with higher fatty acid uptake. Another study of 27 patients with a variety of chronic lung diseases used both 201Tl-thalium (as a marker of myocardial perfusion) and BMIPP imaging (8). The authors
found a positive correlation between severity of PH (as measured by lower RV ejection fraction) and both thalium and BMIPP uptake. A subset of 9 patients with the lowest RV ejection fraction had the greatest thalium uptake, but lower BMIPP uptake than thalium uptake. Finally, a recent study reported an increase in intracardiomyocyte lipid deposition in RV tissue from 2 patients with PAH, a potential marker of dysregulated fatty acid metabolism, along with decreased mRNA of lipid metabolism enzymes including ARV1, CORIN, and PIGK (7).

These small studies of RV metabolism in human disease suggest that fatty acid uptake increases in early or mild PH and then decreases in severe or late PH. It is similarly thought that, in LV failure, in mild disease there is an increase in fatty acid metabolism, which transitions to decreased fatty acid metabolism when the disease becomes severe, along with a concurrent increase in glucose metabolism (3, 17, 20, 23). A biological rationale for a metabolic shift to increased glucose and decreased fatty acid utilization is maximizing oxygen efficiency in generating ATP, since fatty acid β-oxidation requires 12% more O₂ per unit of ATP generated than glucose oxidation (13).

Previous studies of fatty acid metabolism in PH rodent models have had mixed results, likely due to heterogeneity between analysis techniques and models with variable disease severity. The RV tissue of SU-Hx rats, but not PA-banded rats (potentially a more mild form of PH), has previously been reported to show decreased PGC-1α, a key regulator of mitochondrial biogenesis, along with decreased mitochondrial density and abnormal function (6). The authors also found that several fatty acid metabolism genes were decreased in SU-Hx (but not PA-banded) rat RV tissue, including ACSL1, CPT1α, CPT1β, CPT2, ACADS, ACADM, and ACADVL (6), similar to our observations here. As in human PAH, intracardiomyocyte lipid deposition was also found to be present in mice with global BMPR2 mutation (but not PA-banded mice) (7). These BMPR2 mutant mice had increased ceramide and its synthetic enzyme SPT1, potentially contributing to the lipotoxicity (7). In contrast, in Langendorff isolated hearts from PA-banded rats, both fatty acid oxidation and glycolysis were increased as
assessed by metabolism of $[^{14}C]$glucose and $[^{14}C]$palmitate to $[^{14}C]$CO$_2$. In intact PA-banded rats, the fatty acid oxidation inhibitor trimetazidine decreased RV hypertrophy and improved cardiac output, suggesting that increased fatty acid oxidation in this model may be detrimental (5). There may also be a shift to alternative substrates feeding the citric acid cycle, with increased $[^{14}C]$glutamine conversion to $[^{14}C]$CO$_2$ in Langendorf isolated hearts from rats treated with monocrotaline (but not PA banding) (14).

In conclusion, we observed in a model of SPh a shift in RV metabolic substrate, with a significant decrease in fatty acid analog uptake and a mild increase in glucose analog uptake: a phenotype similar to that described in end-stage LV failure.

18F-FTHA and 18F-FDG PET can be used to noninvasively assess changes in substrate uptake in the RV in experimental PH models and may be extendable to human imaging.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


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