Quantifying cardiac sympathetic denervation: first studies of \(^{18}\)F-fluorohydroxyphenethylguanidines in cardiomyopathy patients

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Abstract

Purpose 4-\(^{18}\)F-Fluoro-\(m\)-hydroxyphenethylguanidine (\(^{18}\)F-4F-MHPG) and 3-\(^{18}\)F-fluoro-\(p\)-hydroxyphenethylguanidine (\(^{18}\)F-3F-PHPG) were developed for quantifying regional cardiac sympathetic nerve density using tracer kinetic analysis. The aim of this study was to evaluate their performance in cardiomyopathy patients.

Methods Eight cardiomyopathy patients were scanned with \(^{18}\)F-4F-MHPG and \(^{18}\)F-3F-PHPG. Also, regional resting perfusion was assessed with \(^{13}\)N-ammonia. \(^{18}\)F-4F-MHPG and \(^{18}\)F-3F-PHPG kinetics were analyzed using the Patlak graphical method to obtain Patlak slopes \(K_p\) (mL/min/g) as measures of regional nerve density. Patlak slope polar maps were used to evaluate the pattern and extent of cardiac denervation. For comparison, “retention index” (RI) values (mL blood/min/mL tissue) were also calculated and used to assess denervation. Perfusion polar maps were used to estimate the extent of hypoperfusion.

Results Patlak analysis of \(^{18}\)F-4F-MHPG and \(^{18}\)F-3F-PHPG kinetics was successful in all subjects, demonstrating the robustness of this approach in cardiomyopathy patients. Substantial regional denervation was observed in all subjects, ranging from 25 to 74% of the left ventricle. Denervation zones were equal to or larger than the size of corresponding areas of hypoperfusion. The two tracers provided comparable metrics of regional nerve density and the extent of left ventricular denervation. \(^{18}\)F-4F-MHPG exhibited faster liver clearance than \(^{18}\)F-3F-PHPG, reducing spillover from the liver into the inferior wall. \(^{18}\)F-4F-MHPG was also metabolized more consistently in plasma, which may allow application of population-averaged metabolite corrections.

Conclusion The advantages of \(^{18}\)F-4F-MHPG (more rapid liver clearance, more consistent metabolism in plasma) make it the better imaging agent to carry forward into future clinical studies in patients with cardiomyopathy.

Trial registration: Registered at the ClinicalTrials.gov website (NCT02669563).

Keywords Sympathetic nervous system · Arrhythmia · Sudden cardiac death

Introduction

Sudden cardiac death (SCD) from a precipitous onset of cardiac arrhythmia is a common cause of death in patients with cardiomyopathy [1]. Increasing use of implantable cardioverter defibrillators (ICDs) and cardiac resynchronization therapy (CRT) devices as preventative therapy against SCD has reduced mortality rates in this population [2]. The primary measure used to select patients for ICD therapy is a left ventricular ejection fraction (LVEF) below 35% [3]. Unfortunately, LVEF alone is a poor predictor of who will benefit from ICD therapy, as only about 1 in 8 people who receive an ICD will experience a lifesaving shock [4]. Also,
inappropriate shocks for arrhythmic events that are not life-threatening are not uncommon and can be fatal [5].

Remodeling of cardiac sympathetic innervation occurs in many diseases and has been linked to an increased risk of ventricular arrhythmias, the most frequent arrhythmia to cause SCD [6]. Myocardial infarction causes sympathetic denervation zones which are often larger than the corresponding myocardial scar, demonstrating the sensitivity of sympathetic neurons to ischemic insult [7]. Alterations in ion channel function in denervated myocardium is one mechanism that can promote malignant ventricular arrhythmias [8]. The link between sympathetic remodeling and arrhythmogenesis has led to investigation of noninvasive cardiac denervation metrics, obtained using sympathetic innervation radiotracers, as predictors of SCD risk. The ADMIRE-HF trial with [123I]-meta-iodobenzylguanidine ([123I]-mIBG) established the independent power of the heart-to-mediastinum ratio (H/M ratio) measured with planar scintigraphy, a global measure of cardiac denervation, to predict SCD risk [9]. The PAREPET trial showed that the extent of regional cardiac denervation, measured with [11C]-(−)-m-hydroxyephedrine ([11C]-HED) and PET, classified ischemic cardiomyopathy patients into tertiles with low, medium and high risk of SCD [10]. Also, several clinical studies have shown that higher H/M ratios for [123I]-mIBG and lower denervation extent metrics measured with [11C]-HED are linked to improvements in myocardial function and sympathovagal balance in cardiomyopathy patients treated with CRT devices [11–13]. These findings point to a potential role of cardiac neuroimaging in improved staging of patients for CRT or ICD therapy.

We recently developed two new sympathetic innervation radiotracers, 4-[18F]-fluoro-3-hydroxyphenethylguanidine ([18F]-4F-MHPG) and 3-[18F]-fluoro-p-hydroxyphenethylguanidine ([18F]-3F-PHPG). In addition to the advantages of their fluorine-18 radiolabel, these tracers were designed to have improved kinetics for more accurate quantification of regional nerve density using tracer kinetic analysis techniques. They have slower neuronal uptake rates than [123I]-mIBG and [11C]-HED and much longer neuronal retention times (i.e., they have “irreversible” tissue kinetics) [14]. Slowing neuronal uptake rates reduces flow-limitation effects that occur for tracers with rapid uptake into tissue compartments, which in principle should greatly improve sensitivity for detecting early denervation [15]. Irreversible tissue kinetics affords high tracer tissue concentrations for imaging despite the slower neuronal uptake rates and allows for the application of alternative kinetic analysis techniques. Specifically, studies with [18F]-4F-MHPG and [18F]-3F-PHPG in non-human primates and healthy human subjects showed that the Patlak graphical method [16] provides reproducible metrics of regional nerve density [14, 17]. The goal of this study was to perform a head-to-head comparison of [18F]-4F-MHPG and [18F]-3F-PHPG in cardiomyopathy patients selected for ICD placement to determine which has the best overall properties for measuring cardiac sympathetic denervation using PET.

Methods

Study design

Subjects were scanned with both [18F]-4F-MHPG and [18F]-3F-PHPG. [13N]-Ammonia scans were also performed to evaluate resting perfusion. Eight subjects (8 males, ages 57 ± 15 years, range 37–75 years) were recruited from our population of cardiomyopathy patients staged for ICD or CRT therapy. Inclusion criteria were age 18–80 years, cardiomyopathy (ischemic and nonischemic) and LVEF ≤ 35%. Exclusion criteria included renal dysfunction, pregnancy or lactation, and concomitant medications known to interact with sympathetic nerves. Exclusionary medications were tricyclic antidepressants, cocaine, or medications containing sympathomimetic amines (e.g., phenylephrine), which inhibit the noradrenergic transporter; tetrabenazine or reserpine, which inhibit the vesicular monoamine transporter isofore 2; monoamine oxidase inhibitors; and the centrally acting antihypertensives α-methyldopa and clonidine, which modulate peripheral autonomic function. The study was approved by the University of Michigan Institutional Review Board, and all subjects signed an informed consent document. The study was performed under an exploratory Investigational New Drug clearance from the US Food and Drug Administration and was registered at the ClinicalTrials.gov website (NCT02669563).

Radiotracer production

[18F]-4F-MHPG and [18F]-3F-PHPG were prepared as previously reported, with radiochemical yields of 1.34 ± 0.49 GBq and 1.33 ± 0.36 GBq, respectively. Radiochemical purities averaged 97.2 ± 3.7% (range 90.7–100%) for [18F]-4F-MHPG and 99.1 ± 1.1% (range 98.5–100%) for [18F]-3F-PHPG. Molar activities averaged 109 ± 54 GBq/μmol for [18F]-4F-MHPG and 184 ± 142 GBq/μmol for [18F]-3F-PHPG.

PET/CT data acquisition and processing

Scans were performed on either a Siemens Biograph mCT scanner (n = 4) or a Siemens Biograph TruePoint TrueV scanner (n = 4), which have the same detector block design and equivalent spatial resolution. All scans for a subject were done on the same scanner. On one day, an [13N]-ammonia scan was performed (740 MBq, 20-min dynamic imaging) followed 1 h later by an [18F]-4F-MHPG or [18F]-3F-PHPG...
The other innervation tracer was done on a different day. During innervation scans, six venous blood samples (2 mL each) were drawn to determine tracer metabolism in plasma and partitioning of activity between plasma and red blood cells, as previously described [17]. 18F-4F-MHPG doses of averaged 255 ± 2 MBq (masses 0.74 ± 0.31 μg; range 0.29–1.29 μg). 18F-3F-PHPG injections averaged 253 ± 5 MBq (masses 0.66 ± 0.55 μg; range 0.22–1.72 μg).

Dynamic PET data (60 min) were binned into a 24-frame sequence (12 × 10, 2 × 30, 2 × 60, 2 × 150, 2 × 300, and 4 × 600 s). No cardiac gating or cardiac motion corrections were performed. Images were reconstructed using iterative OSEM with 4 iterations and 14 subsets (TruePoint scanner) or 4 iterations and 12 subsets (mCT scanner) and smoothed with a 5-mm Gaussian filter.

Image analysis

After image reorientation, short-axis images were interpolated into 4-mm slices (PMOD v3.8, PMOD Technologies). The left ventricular wall on the final frame was divided into 60 sectors using an algorithm written in IDL (v6.2, Harris Geospatial Solutions). Using 18 short-axis slices encompassing the left ventricle, 18 × 60 = 1080 volumes-of-interest (VOIs) were defined, and tissue time-activity curves $C_t(t)$ were extracted for each VOI. A whole-blood time-activity curve $C_{wb}(t)$ was obtained from a VOI placed in the basal left ventricular blood pool.

Blood sample analysis

Blood sample times were $t = 2, 5, 8, 15, 30$, and 50 min for 18F-4F-MHPG and $t = 4, 8, 12, 20, 35$, and 50 min for

| Subject | Age (years) | Weight (kg) | BMI | NYHA class | LVEF (%) | MI | HTN | AF | ICM | NICM | DM T2 | Prior RV | Implanted ICD Device |
|---------|-------------|-------------|-----|------------|----------|----|-----|----|-----|------|-------|----------|----------------------|
| 1       | 74          | 120         | 34  | III        | 18       | ✓  | ✓   | ✓  |      |      |       | CABG×4               | Biventricular with CRT-D |
| 2       | 39          | 138         | 46  | II         | 15       | ✓  |      | ✓  |      |      |       | CABG×3               | Single chamber          |
| 3       | 60          | 104         | 35  | I          | 25       | ✓  | ✓   | ✓  |      |      |       | PCI                  | Dual chamber            |
| 4       | 67          | 78          | 23  | I          | 35       | ✓  | ✓   | ✓  |      |      |       | PCI                  | Single chamber          |
| 5       | 37          | 85          | 27  | I          | 22       | ✓  | ✓   | ✓  |      |      |       | Single chamber        |                       |
| 6       | 43          | 80          | 28  | III        | 18       | ✓  |      | ✓  |      |      |       | CABG×3               | Single chamber          |
| 7       | 61          | 79          | 24  | I          | 25       | ✓  | ✓   | ✓  |      |      |       | PCI                  | Single chamber          |
| 8       | 73          | 109         | 31  | I          | 21       | ✓  |      | ✓  |      |      |       | Single chamber        |                       |

BMI, body mass index; NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; MI, myocardial infarction; HTN, hypertension; AF, atrial fibrillation; ICM, ischemic cardiomyopathy; NICM, nonischemic cardiomyopathy; DM T2, diabetes mellitus type 2; RV, revascularization; CABG, coronary artery bypass grafting; PCI, percutaneous coronary intervention; ICD, implantable cardioverter defibrillator; CRT-D, cardiac resynchronization therapy defibrillator.

Scan. The other innervation tracer was done on a different day. During innervation scans, six venous blood samples (2 mL each) were drawn to determine tracer metabolism in plasma and partitioning of activity between plasma and red blood cells, as previously described [17]. 18F-4F-MHPG doses of averaged 255 ± 2 MBq (masses 0.74 ± 0.31 μg; range 0.29–1.29 μg). 18F-3F-PHPG injections averaged 253 ± 5 MBq (masses 0.66 ± 0.55 μg; range 0.22–1.72 μg). Dynamic PET data (60 min) were binned into a 24-frame sequence (12 × 10, 2 × 30, 2 × 60, 2 × 150, 2 × 300, and 4 × 600 s). No cardiac gating or cardiac motion corrections were performed. Images were reconstructed using iterative OSEM with 4 iterations and 14 subsets (TruePoint scanner) or 4 iterations and 12 subsets (mCT scanner) and smoothed with a 5-mm Gaussian filter.
Radio-HPLC analysis of purified plasma aliquots (100–300 μL) determined the fraction of plasma activity associated with intact radiotracer, $f_{\text{intact}}(t)$. The $f_{\text{intact}}(t)$ vs. time data were fit to dose–response functions using GraphPad Prism v3.03 as previously described [18]. Whole-blood and plasma aliquots (100 μL) were counted in a gamma counter to measure the plasma to whole-blood activity concentration ratio, $C_p/C_{wb}$. This tended to be constant during a study, so the mean ratio $C_p/C_{wb}$ was calculated. A plasma time-activity curve, $C_p(t)$, was estimated from the whole-blood curve as: $C_p(t) = f_{\text{intact}}(t) \cdot (C_p/C_{wb}) \cdot C_{wb}(t)$.

**Tracer kinetic analysis**

Kinetic analyses were performed using PMOD’s Kinetic Modeling (PKIN) module. For $^{18}$F-4F-MHPG and $^{18}$F-3F-PHPG, Patlak plots were constructed from the tissue and plasma time-activity curves and analyzed with linear regression to generate regional Patlak slopes, $K_p$ (mL/min/g), and the linear regression coefficient $r$, as previously described [17]. Regression analysis used data from the 4.5 min frame to the final frame. $^{13}$N-Ammonia kinetics were analyzed using the DeGrado method [19] to obtain regional perfusion estimates, $F$ (mL/min/g). Using polar maps of the Patlak slopes or perfusion estimates, the fraction of VOIs with nerve density or perfusion estimates less than a cutoff threshold of 50% of the maximum map value were calculated to estimate the extent of left ventricular denervation or hypoperfusion.

**Tracer retention measures**

For comparison with Patlak analysis, tracer “retention index” (RI) metrics for $^{18}$F-4F-MHPG and $^{18}$F-3F-PHPG were calculated by dividing the final tracer tissue concentration $C_t$ in each VOI by the integral of the whole-blood time-activity curve, $C_{wb}(t)$, using the Tracer Retention algorithm in the PKIN module of PMOD. The fraction of VOIs with RI values less than a cutoff threshold of 50% of the maximum RI was calculated to estimate the extent of cardiac denervation.

**Safety and tolerability tests**

Safety tests of $^{18}$F-4F-MHPG and $^{18}$F-3F-PHPG were performed before and after each PET session, including a resting 12-lead ECG, and measurements of heart rate, blood pressure, respiration, and body temperature. Blood tests (comprehensive metabolic panel, complete blood count, plasma catecholamine levels) and urinalysis were also performed. Heart rate and peripheral capillary oxygen saturation (SpO2) were continuously monitored during PET scanning, and blood pressure measured every 10 min. Subjects were contacted at 24 h and 30 days to enquire about any adverse events.

**Statistical analysis**

Statistical calculations were performed using Microsoft Excel 2016.
Results

Subject characteristics

Clinical data for the 8 subjects are summarized in Table 1. Six subjects had ischemic cardiomyopathy with prior myocardial infarction. The other two had nonischemic cardiomyopathy with no known infarctions. None had mixed etiology. Single-chamber ICD devices were most often implanted, while one was a dual-chamber ICD, and another was a biventricular ICD with a cardiac resynchronization therapy defibrillator (CRT-D). Concomitant medications included the statin atorvastatin \((n = 6)\); the selective serotonin receptor inhibitor (SSRI) citalopram \((n = 1)\); the mixed nonselective beta-blocker and \(\alpha_1\)-adrenoreceptor antagonist carvedilol \((n = 4)\) or the \(\beta_1\)-adrenoreceptor antagonist metoprolol \((n = 3)\); the angiotensin II type 1 receptor (AT\(_1\)) antagonist losartan \((n = 2)\); and the angiotensin-converting enzyme (ACE) inhibitors lisinopril \((n = 5)\) or ramipril \((n = 1)\).

Safety

\(^{18}\)F-4F-MHPG and \(^{18}\)F-3F-PHPG were well tolerated in all subjects. No significant changes in vital signs were observed during PET scanning, and there were no effects on ECG data or laboratory test results. No adverse events were reported.

PET Imaging

Seven of the eight subjects completed all three PET scans. One subject (\#1) ended their innervation scans early \((at t = 40\) min\) due to back pain. Another subject (\#3) left the study after completing scans with \(^{13}\)N-ammonia and \(^{18}\)F-4F-MHPG. Representative fused PET/CT scans are shown in Fig. 1. \(^{18}\)F-4F-MHPG and \(^{18}\)F-3F-PHPG produced high-quality cardiac PET images, with low lung uptake and good heart-to-blood contrast. More prolonged retention of \(^{18}\)F-3F-PHPG in the liver relative to \(^{18}\)F-4F-MHPG is evident in the coronal images.

Tissue concentration ratios

Peak heart uptake levels and mean concentrations in blood, liver, and lung were determined in the last five image frames to calculate the tissue ratios: peak heart-to-liver, peak heart-to-blood, and peak heart-to-lung (Fig. 2). \(^{18}\)F-4F-MHPG exhibited significantly higher peak heart-to-liver ratios at all times, increasing from \(1.57 \pm 0.29\) at 17.5 min to \(2.55 \pm 0.35\) at 55 min, compared with \(0.75 \pm 0.25\) to \(1.15 \pm 0.40\) for \(^{18}\)F-3F-PHPG (Fig. 2A; paired \(t\)-test \(p < 0.007\) at all times, range \(0.0005\) to \(0.007\)). Peak heart-to-liver ratios for \(^{18}\)F-4F-MHPG over those of \(^{18}\)F-3F-PHPG were consistently 2.2 times higher \((2.2 \pm 0.1)\). Conversely, \(^{18}\)F-3F-PHPG had significantly higher peak heart-to-blood ratios at all times, with final frame values of \(13.4 \pm 6.7\) compared to \(7.6 \pm 2.8\) for \(^{18}\)F-4F-MHPG (Figs. 2B; \(p < 0.04\) at all times, range \(0.003\) to \(0.04\)), averaging 1.8 times higher \((1.8 \pm 0.1)\). \(^{18}\)F-3F-PHPG also had consistently higher peak heart-to-lung ratios (Fig. 2C), averaging 40% higher than those of \(^{18}\)F-4F-MHPG \((1.4 \pm 0.0)\). This was significant from 17.5 to 45 min \((p < 0.007\) to 0.02), but not at 55 min \((p < 0.09)\).

Blood sample analysis

Metabolism of \(^{18}\)F-4F-MHPG and \(^{18}\)F-3F-PHPG in plasma was biphasic (Fig. 3). A large fraction of \(^{18}\)F-4F-MHPG is rapidly metabolized in the early phase, with times of 50%
metabolized ($T_{50\%}$) averaging $5.7 \pm 1.1$ min. $^{18}$F-3F-PHPG metabolism was significantly slower (paired two-tailed $t$-test $p < 0.01$) and more variable, with $T_{50\%}$ values averaging $11.2 \pm 4.3$ min. Plasma to whole blood ratios, $C_p/C_{wb}$, were consistent within a given subject, ranging from $1.32 \pm 0.10$ to $1.82 \pm 0.15$ for $^{18}$F-4F-MHPG and from $1.34 \pm 0.06$ to $1.67 \pm 0.09$ for $^{18}$F-3F-PHPG. Across subjects, $C_p/C_{wb}$ averaged $1.45 \pm 0.18$ for $^{18}$F-4F-MHPG and $1.48 \pm 0.15$ for $^{18}$F-3F-PHPG.

**Tracer kinetic analysis**

Patlak analysis of $^{18}$F-4F-MHPG and $^{18}$F-3F-PHPG kinetics was successful in all regions for all subjects. Figure 4B shows examples of $^{18}$F-3F-PHPG kinetics in regions covering a range of nerve densities. Corresponding Patlak plots are shown in Fig. 4C. Highly linear fits of the Patlak plot data were obtained, independent of the degree of denervation. Linear correlation coefficients $r$ (mean ± SD within a subject) ranged from $0.965 \pm 0.025$ to $0.995 \pm 0.003$ for $^{18}$F-4F-MHPG and from $0.990 \pm 0.009$ to $0.995 \pm 0.005$ for $^{18}$F-3F-PHPG. Patlak slopes and resting perfusion estimates are presented in Table 2. The distribution of Patlak slopes in each subject is illustrated in Fig. 5. All subjects had some regions with Patlak slopes in the normal range, representing regions with preserved innervation, as well as regions with Patlak slopes below normal, consistent with partial or complete denervation. Within subjects, regional Patlak slopes for $^{18}$F-3F-PHPG and $^{18}$F-4F-MHPG were highly correlated, with linear correlation coefficients $r$ ranging from 0.911 to 0.985 (data not shown). Estimates of the extent of left ventricular hypoperfusion and sympathetic denervation from threshold analysis of the polar map data are provided in Table 3. Polar map examples are shown in Fig. 6. Denervation extent measures for $^{18}$F-4F-MHPG and $^{18}$F-3F-PHPG closely agreed in most subjects, and differed by <5% for all subjects. Correlations between regional Patlak slopes and resting perfusion estimates varied considerably across subjects, with $R^2$ values ranging from 0.123 up to 0.728 (Fig. 7).

**Tracer retention analysis**

Retention index (RI) values and denervation extent estimates obtained using RI values are shown in Table 4. Within individuals, there was strong linear correlation between regional RI values and Patlak slopes, with linear correlation coefficients $r > 0.991$ in all cases for both tracers. This is expected since areas with higher Patlak slopes will generate higher amounts of tracer trapped in neurons. However, the slopes of the correlations were variable across subjects, ranging widely from 0.41 to 0.89 for $^{18}$F-4F-MHPG and from 0.64 to 1.11 for $^{18}$F-3F-PHPG. These correlation slopes should depend largely on the rate of tracer metabolism in each individual, since RI values are normalized to the integral of the whole-blood time-activity curve, uncorrected for metabolites. Correlation slopes should be lower in subjects with faster tracer metabolism, because rapid metabolism leads to less retention of tracer in neurons.

![Fig. 4](image_url)

Fig. 4 A $^{18}$F-3F-PHPG image for Subject #1 showing VOI locations of representative kinetic data. B $^{18}$F-3F-PHPG kinetics in regions with different sympathetic nerve densities, from normal (red) down to severe denervation (violet). C Corresponding Patlak plots for the kinetic data shown in (B), demonstrating the decline of the Patlak slope with decreasing nerve density. VOI = volume-of-interest
Regression analysis with two metrics of tracer metabolism rates supports this. First, the RI vs. $K_p$ correlation slopes and half-times of tracer metabolism (Fig. 3) were significantly correlated, with $R^2 = 0.561$ ($p < 0.03$) and $R^2 = 0.956$ ($p < 0.0001$) for $^{18}$F-4F-MHPG and $^{18}$F-3F-PHPG, respectively. The slopes were also significantly correlated with the area under the metabolism curve (numerical integration, GraphPad Prism v3.03), with $R^2 = 0.686$ ($p < 0.01$) for $^{18}$F-4F-MHPG and $R^2 = 0.918$ ($p < 0.0007$) for $^{18}$F-3F-PHPG. Linear regression of the mean RI value vs. mean Patlak slope for each subject gave $RI = (0.533)K_p + 0.004$ for $^{18}$F-4F-MHPG, which was not significant ($R^2 = 0.368, p < 0.1$). For $^{18}$F-3F-PHPG, the relationship was $RI = (1.282)K_p - 0.035$, which reached significance ($R^2 = 0.671, p < 0.02$). The higher slope of the relationship for $^{18}$F-3F-PHPG is also tied to its slower metabolism. Finally, denervation extent metrics from RI values were in close agreement with those obtained from Patlak slopes (Fig. 8A, B). Strong linear correlations between the two denervation extent metrics were seen for both tracers ($R^2 = 0.996$ ($p < 2 \times 10^{-8}$) for $^{18}$F-4F-MHPG and $R^2 = 0.995$ ($p < 5 \times 10^{-7}$) for $^{18}$F-3F-PHPG.

**Table 2** Global parameter estimates from tracer kinetic analysis

| Subject # | $^{13}$N-Ammonia Resting perfusion (mL/min/g) | CV (%) | $^{18}$F-4F-MHPG Patlak slopes (mL/min/g) | CV (%) | $^{18}$F-3F-PHPG Patlak slopes (mL/min/g) | CV (%) |
|-----------|-----------------------------------------------|--------|------------------------------------------|--------|------------------------------------------|--------|
| 1         | 0.468±0.126                                   | 29.4%  | 0.0632±0.0255                           | 40.3%  | 0.0710±0.0346                           | 48.7%  |
| 2         | 0.522±0.168                                   | 32.2%  | 0.0542±0.0237                           | 43.7%  | 0.0586±0.0268                           | 45.7%  |
| 3         | 0.446±0.215                                   | 48.2%  | 0.0419±0.0256                           | 61.1%  | —                                        | —      |
| 4         | 0.690±0.139                                   | 20.1%  | 0.0626±0.0285                           | 45.5%  | 0.0766±0.0374                           | 48.8%  |
| 5         | 0.633±0.197                                   | 31.1%  | 0.0930±0.0293                           | 31.5%  | 0.1009±0.0342                           | 33.9%  |
| 6         | 0.630±0.174                                   | 27.6%  | 0.0554±0.0267                           | 48.2%  | 0.0675±0.0344                           | 50.9%  |
| 7         | 0.516±0.210                                   | 40.7%  | 0.0696±0.0259                           | 37.2%  | 0.0942±0.0384                           | 40.8%  |
| 8         | 0.388±0.105                                   | 27.1%  | 0.0754±0.0210                           | 27.8%  | 0.0863±0.0237                           | 27.5%  |

Values are mean ± SD. CV coefficient of variation.
Discussion

First-in-human studies of $^{18}$F-4F-MHPG and $^{18}$F-3F-PHPG in healthy subjects demonstrated that their irreversible kinetics can be analyzed with Patlak analysis to estimate regional cardiac sympathetic nerve density, with Patlak slopes averaging $0.107 \pm 0.010 \text{ mL/min/g}$ and $0.116 \pm 0.010 \text{ mL/min/g}$, respectively [17]. The first studies of these tracers in cardiomyopathy patients reported here show they can also provide nerve density measures in hearts with extensive disease-induced denervation. Their administration caused no changes in blood pressure, heart rate, ECG measures, or laboratory tests and caused no adverse events, further establishing their safety.

Comparing $^{18}$F-4F-MHPG and $^{18}$F-3F-PHPG, each agent demonstrated an advantage over the other. $^{18}$F-4F-MHPG had much higher heart-to-liver ratios due to its faster clearance from the liver, providing better heart-to-background contrast. More importantly, the faster liver clearance of $^{18}$F-4F-MHPG reduces spillover of liver counts into adjacent ventricular regions, which can confound Patlak analysis in those regions (Figs. 1 and 2). $^{18}$F-3F-PHPG had consistently higher heart-to-blood ratios, due to moderately lower blood concentrations and slower metabolism in plasma which increased myocardial concentrations. $^{18}$F-3F-PHPG metabolism in plasma was not only slower than $^{18}$F-4F-MHPG, it was also much more variable (Fig. 3). The cause of this is unknown, but could be related to medication effects in the liver. A potential advantage of the more consistent metabolism of $^{18}$F-4F-MHPG is that it may allow the application of population-averaged metabolite corrections for generating input functions, eliminating the need for blood sampling. In kinetic analyses, regional Patlak slopes obtained with $^{18}$F-4F-MHPG and $^{18}$F-3F-PHPG were highly correlated in all subjects and there was also very good agreement between each tracer’s estimate of the extent of left ventricular denervation (Table 3). Thus, the two tracers are essentially equivalent in their ability to quantify regional nerve densities.

$^{18}$F-4F-MHPG and $^{18}$F-3F-PHPG accumulate in cardiac sympathetic nerve varicosities as substrates of the norepinephrine transporter (NET) and are rapidly stored in norepinephrine storage vesicles by the second isoform of the vesicular monoamine transporter (VMAT2). Efficient retention in storage vesicles is the mechanism responsible for their irreversible tissue kinetics [15]. Our results show that $^{18}$F-4F-MHPG and $^{18}$F-3F-PHPG retain their irreversible kinetics in patients with cardiomyopathy,
including cardiac regions with severe nerve losses. This is an important finding since vesicular storage function is an energy-dependent process that can be compromised during acute ischemia [20]. The observation that $^{18}$F-4F-MHPG and $^{18}$F-3F-PHPG maintain their irreversible kinetics in regions with substantial nerve losses indicates that vesicular storage function remains intact in the surviving neurons of patients with chronic cardiomyopathy.

All subjects had regions with Patlak slopes in the normal range (Fig. 5) and the myocardial kinetics of the tracers were irreversible, evidence that concomitant medications did not interfere with tracer uptake and storage. Statins like atorvastatin may reduce the elevated sympathetic outflow seen in cardiomyopathy and can restore sympathovagal balance [21]. Thus, statins may reduce extraneuronal norepinephrine concentrations via reduced outflow. The SSRI citalopram has a binding affinity of only 4.1 mM for NET [22]. At typical blood levels of 0.1–0.7 mg/L [23], equal to concentrations of 0.3 μM–2.1 μM, citalopram should not interfere with cardiac NET transport. The beta-blockers carvedilol and metoprolol do not possess intrinsic sympathomimetic activity and primarily act to inhibit post-synaptic responses to norepinephrine [24]. Also, they do not cause large changes in sympathetic outflow [25], so they should not inhibit NET transport. Supporting this, a meta-analysis of the ADMIRE-HF trial data found that H/M ratios for $^{123}$I-MIBG were independent of beta-blocker dose and plasma norepinephrine levels [26]. The renin–angiotensin–aldosterone system is activated in patients with chronic cardiomyopathy and can restore sympathovagal balance [11]. The one CRT-treated patient in our study had VOIs with Patlak slopes in the normal range at baseline, indicating intact tracer uptake mechanisms in those regions. However, it is possible that nerves in other regions with below normal Patlak slopes could have reduced neuronal function that would improve in response to CRT. Studies with $^{123}$I-mIBG and $^{11}$C-HED have shown that most highly preserved sympathetic innervation at baseline predicts better responsiveness to CRT [12, 13]. Also, in CRT-responsive patients, $^{123}$I-mIBG exhibits higher late H/M ratios and slower washout rates than at baseline, along with better autonomic balance, consistent with improved sympathetic function [11]. The one CRT-treated patient in our study had VOIs with Patlak slopes in the normal range at baseline, indicating intact tracer uptake mechanisms in those regions. However, it is possible that nerves in other regions with below normal Patlak slopes could have reduced neuronal function that would improve in response to CRT. Future studies with $^{18}$F-4F-MHPG or $^{18}$F-3F-PHPG in CRT patients could potentially identify the specific regions that functionally improve, providing insights into CRT-induced changes in presynaptic NET and VMAT2 function.

An advantage of Patlak analysis is it only requires linear regression of the transformed kinetic data, a robust method that can be applied to small region sizes. In preclinical studies in nonhuman primates with $^{18}$F-4F-MHPG, Patlak slopes showed good reproducibility (± 10% or less) under control conditions [14]. Also, in pharmacological blocking studies using different doses of the potent NET inhibitor desipramine (DMI) to establish varying fractions of available cardiac NET transporters, Patlak slopes declined with increasing DMI doses following a sigmoidal dose–response model, showing that they sensitively tracked declines in available NET. At the highest dose of DMI (1.0 mg/kg), blood activity exceeded myocardial activity, consistent with

### Table 4 Calculated retention index values and estimated innervation defect sizes

| Subject | $^{18}$F-4F-MHPG RI (mL blood/min/mL tissue) | $^{18}$F-4F-MHPG CV (%) | $^{18}$F-3F-PHPG RI (mL blood/min/mL tissue) | $^{18}$F-3F-PHPG CV (%) | $^{18}$F-4F-MHPG (% denervated) | $^{18}$F-3F-PHPG (% denervated) |
|---------|---------------------------------------------|------------------------|---------------------------------------------|------------------------|-------------------------------|-------------------------------|
| 1       | 0.0386 ± 0.0148                             | 38.3%                  | 0.0576 ± 0.0268                             | 46.5%                  | 57.6%                         | 62.2%                         |
| 2       | 0.0363 ± 0.0157                             | 43.3%                  | 0.0381 ± 0.0174                             | 45.7%                  | 70.9%                         | 64.3%                         |
| 3       | 0.0220 ± 0.0133                             | 60.5%                  | –                                           | –                      | 73.7%                         | –                             |
| 4       | 0.0263 ± 0.0118                             | 44.9%                  | 0.0649 ± 0.0315                             | 48.5%                  | 56.7%                         | 63.1%                         |
| 5       | 0.0414 ± 0.0128                             | 30.9%                  | 0.1021 ± 0.0349                             | 34.2%                  | 37.7%                         | 34.4%                         |
| 6       | 0.0339 ± 0.0152                             | 44.8%                  | 0.0498 ± 0.0243                             | 48.8%                  | 63.1%                         | 63.1%                         |
| 7       | 0.0433 ± 0.0160                             | 37.0%                  | 0.0617 ± 0.0245                             | 39.7%                  | 41.6%                         | 40.2%                         |
| 8       | 0.0670 ± 0.0188                             | 28.1%                  | 0.0968 ± 0.0264                             | 27.3%                  | 27.6%                         | 24.4%                         |

Values are mean ± SD. RI retention index, CV coefficient of variation.
complete blockade of NET, demonstrating the high specificity of $^{18}$F-4F-MHPG for sympathetic nerves.

The side-by-side presentation of perfusion and innervation polar maps (Fig. 6) and the plots of regional innervation metrics vs. perfusion estimates (Fig. 7) illustrate how nuclear cardiologists could compare the pattern and extent of sympathetic denervation with regional perfusion to assess SCD risk. The observed patterns between regional nerve density and perfusion depend on the complex history of the progression of each subject’s cardiovascular disease and treatments. For example, in some subjects, the polar maps showed that revascularization had restored perfusion to areas that remain denervated (e.g., Fig. 6A and B). Zones of denervated myocardium that are well perfused (“perfusion-innervation mismatch” zones) are areas that promote the genesis of ventricular arrhythmias [29]. In this study, five of the eight subjects had denervation zones that were larger than the area of hypoperfusion (Table 3). All five had ischemic cardiomyopathy with prior infarctions. Conversely, two subjects with matched hypoperfusion and denervation zones had nonischemic cardiomyopathy and no known infarctions. Whether this pattern difference between ischemic and nonischemic cardiomyopathies would be a consistent finding in a larger cohort remains to be seen, since the PAREPET trial excluded patients with nonischemic cardiomyopathy. However, PAREPET evaluated a perfusion-innervation mismatch metric which demonstrated power in predicting arrhythmic risk, but the denervation extent measure alone was found to be a stronger predictor of sudden cardiac arrest [10].

Clinical studies with $^{123}$I-mIBG and $^{11}$C-HED rely on semi-quantitative metrics of tracer retention, such as the H/M ratio for $^{123}$I-mIBG or RI values for $^{11}$C-HED, as measures of global or regional sympathetic nerve density, respectively [7, 9]. While these measures have served as useful first approaches to quantifying nerve losses, a fluorine-18 PET radiotracer that can accurately and reproducibly quantify regional nerve density using kinetic analysis methods is highly desirable, not only for routine clinical studies but also for research on the impact of diseases or new therapeutics on cardiac nerve populations [30]. In this study, we compared nerve density and denervation extent measures obtained using Patlak slopes and RI values. While regional RI values were highly correlated with regional Patlak slopes within individual subjects, the slopes of these correlations varied, due to variation in tracer metabolism rates. Because individual tracer metabolism rates are unaccounted for in the RI calculation, the variation in RI values across individuals limits their utility as nerve density metrics on an absolute scale. However, denervation extent estimates from RI values were within a few percent of those obtained from the Patlak slope data (Fig. 8). Therefore, if the sole purpose of a clinical PET study with $^{18}$F-4F-MHPG or $^{18}$F-3F-PHPG is to estimate the extent of cardiac denervation, RI data would be adequate for this purpose and would avoid blood sample analysis. On the other hand, if the goal was to obtain quantitative measures of regional nerve density in addition to the denervation extent measure, Patlak analysis would be the method of choice.
Limitations of this study include the small number of subjects and the absence of female subjects in the study cohort, which limits its generalizability to all cases of cardiomyopathy. Future studies will include a similar head-to-head study design comparing the performance of $^{18}$F-4F-MHPG with $^{11}$C-HED for quantifying the extent of left ventricular denervation.

**Conclusion**

In conclusion, $^{18}$F-4F-MHPG and $^{18}$F-3F-PHPG can each be used to quantify sympathetic nerve density at high regional resolution using Patlak analysis, a unique attribute that distinguishes them from existing cardiac innervation tracers. Nerve density and denervation extent measures obtained with the two agents were comparable. The strengths of $^{18}$F-4F-MHPG include its more rapid clearance from the liver, reducing spillover from the liver into the inferior wall, and its more consistent metabolism in plasma, which may make it possible to avoid blood sampling by using a population-averaged metabolite correction curve. These two advantages outweigh the higher heart-to-blood contrast of $^{18}$F-3F-PHPG, making $^{18}$F-4F-PHPG the better agent to carry forward for further clinical development. Our results support the performance of larger clinical trials to establish the power of nerve density and denervation extent metrics obtained with $^{18}$F-4F-MHPG to predict clinical outcomes in patients being evaluated for CRT or ICD therapy.

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**Declarations**

**Ethical approval** The study was approved by the University of Michigan Institutional Review Board (HUM00105110). This article does not contain any studies with animals performed by any of the authors. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

**Conflict of interest** The authors declare that they have no conflict of interest.

**References**

1. Adabag AS, Luepker RV, Roger VL, Gersh BJ. Sudden cardiac death: epidemiology and risk factors. Nat Rev Cardiol. 2010;7:216–25.
2. Saour B, Smith B, Yancy CW. Heart failure and sudden cardiac death. Card Electrophysiol Clin. 2017;9:709–23.
3. Buxton AE, Waks JW, Chen CS, Chen PS. Risk stratification for sudden cardiac death in North America – current perspectives. J Electrocardiol. 2016;49:817–23.
4. Passman R, Goldberg JJ. Predicting the future: risk stratification for sudden cardiac death in patients with left ventricular dysfunction. Circulation. 2012;125:3013–37.
5. Koneru JN, Swerdlow CD, Wood MA, Ellenbogen KA. Minimizing inappropriate or “unnecessary” implantable cardioverter-defibrillator shocks: appropriate programming. Circ Arrhythm Electrophysiol. 2011;4:778–90.
6. Srinivasa NT, Schilling RJ. Sudden cardiac death and arrhythmias. Arrhythm Electrophysiol Rev. 2018;7:111–7.
7. Allman KC, Wieland DM, Muzik O, Degrado TR, Wolfe ER, Schweiger M. Carbon-11 hydroxyephedrine with positron emission tomography for serial assessment of cardiac adrenergic neuronal function after acute myocardial infarction in humans. J Am Coll Cardiol. 1993;22:368–75.
8. Gardner RT, Ripplinger CM, Myles RC, Habeker BA. Molecular mechanisms of sympathetic remodeling and arrhythmias. Circ Arrhythm Electrophysiol. 2016;9:e001359.
9. Jacobson AF, Senior R, Cerequeira MD, Wong ND, Thomas GS, Lopez VA, et al. Myocardial iodine-123 meta-iodobenzylguanidine imaging and cardiac events in heart failure: results of the prospective ADMIRE-HF (AdreView Myocardial Imaging for Risk Evaluation in Heart Failure) study. J Am Coll Cardiol. 2010;55:2212–21.
10. Fallavollita JA, Heavey BM, Luisi AJ, Michalek SM, Baldwa S, Mshatere TL, et al. Regional myocardial sympathetic denervation predicts the risk of sudden cardiac arrest in ischemic cardiomyopathy. J Am Coll Cardiol. 2014;63:141–9.
11. Cha YM, Chareonthaitawee P, Dong YX, Kemp BJ, Oh JK, Miyazak IC, et al. Cardiac sympathetic reserve and response to cardiac resynchronization therapy. Circ Heart Fail. 2011;4:339–44.
12. Martignani C, Diemberger I, Nanni C, Biffi M, Ziacchi M, Boschi S, et al. Cardiac resynchronization therapy and cardiac sympathetic function. Eur J Clin Invest. 2015;45:792–9.
13. Verschure DO, Poel E, De Vincentis G, Frantellizzi V, Nakajima K, Gheyssens O, et al. The relation between cardiac $^{18}$F-mIBG scintigraphy and functional response 1 year after CRT implantation. Eur Heart J Cardiovasc Imaging. 2021;22:49–57.
14. Jang KS, Jung YW, Gu G, Koepp RA, Sherman PS, Quesada CA, et al. 4-[$^{18}$F]fluoro-m-hydroxyphenethylguanidine: a radiopharmaceutical for quantifying regional cardiac sympathetic nerve density with positron emission tomography. J Med Chem. 2013;56:7312–23.
15. Raffel DM, Jung YW, Gildersleeve DL, Sherman PS, Moskwa JJ, Tluczek LJ, et al. Radiolabeled phenethylguanidines: novel imaging agents for cardiac sympathetic neurons and adrenergic tumors. J Med Chem. 2007;50:2078–88.
16. Patlak CS, Blasberg RG. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. Generalizations J Cereb Blood Flow. 1985;5:584–90.
17. Raffel DM, Jung YW, Koeppe RA, Jang KS, Gu G, Scott PJH, et al. First-in-human studies of \([^{18}F]\)fluorohydroxyphenethylguanidines: positron emission tomography radiotracers for quantifying regional cardiac sympathetic nerve density. Circ Cardiovasc Imaging. 2018;11:e007965.

18. Raffel DM, Koeppe RA, Jung YW, Gu G, Jang KS, Sherman PS, et al. Quantification of cardiac sympathetic nerve density with \(N^{11}C\)-guanyl-meta-octopamine and tracer kinetic analysis. J Nucl Med. 2013;54:1645–52.

19. DeGrado TR, Hanson MW, Turkington TG, Delong DM, Brezinski DA, Vallée JP, et al. Estimation of myocardial blood flow for longitudinal studies with \(^{15}N\)-labeled ammonia and positron emission tomography. J Nucl Cardiol. 1996;3:494–507.

20. Kurz T, Richardt G, Hagl S, Seyfarth M, Schömig A. Two different mechanisms of noradrenaline release during normoxia and simulated ischemia in human cardiac tissue. J Mol Cell Cardiol. 1995;27:1161–72.

21. Lewandowski J, Symonides B, Gaciong Z, Sinski M. The effect of statins on sympathetic activity: a meta-analysis. Clin Auton Res. 2014;25:125–31.

22. Tatsumi M, Groshan K, Blakely RD, Richelson E. Pharmacological profile of antidepressants and related compounds at human monoamine transporters. Eur J Pharmacol. 1997;340:249–58.

23. Reis M, Aamo T, Ahlner J, Druid H. Reference concentrations of antidepressants. a compilation of postmortem and therapeutic levels. J Anal Toxicol. 2007;31:254–64.

24. Frishman WH, Saunders E. \(\beta\)-Adrenergic blockers. J Clin Hypertension. 2011;13:649–53.

25. Tank J, Diedrich A, Schroeder C, Stoffels M, Franke G, Sharma AM, et al. Limited effect of systemic \(\beta\) blockade on sympathetic outflow. Hypertension. 2001;38:1377–81.

26. Cohen-Solal A, Jacobson AF, Piña IL. Beta blocker dose and markers of sympathetic activation in heart failure patients: interrelationships and prognostic significance. ESC Heart Failure. 2017;4:499–506.

27. Krum H. Differentiation in the angiotensin II receptor 1 blocker class on autonomic function. Curr Hypertens Rep. 2001;3(Suppl 1):S17–23.

28. Miller AJ, Arnold AC. The renin–angiotensin system in cardiovascular autonomic control: recent developments and clinical implications. Clin Auton Res. 2019;29:231–43.

29. Simões MV, Barthel P, Matsunari I, Nekolla SG, Schömig A, Schwaiger M, et al. Presence of sympathetically denervated but viable myocardium and its electrophysiologic correlates after early revascularised, acute myocardial infarction. Eur Heart J. 2004;25:551–7.

30. Bengel FM. Imaging of myocardial catecholamine uptake: toward robust absolute quantification [editorial]. Circ Cardiovasc Imaging. 2018;11:e008534.

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