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Rapid dual-injection single-scan ^{13}N -ammonia PET for quantification of rest and stress myocardial blood flows

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Abstract

Quantification of myocardial blood flows at rest and stress using ^{13}N -ammonia PET is an established method; however, current techniques require a waiting period of about one hour between scans. The objective of this study was to test a rapid dual-injection single-scan approach, where ^{13}N -ammonia injections are administered 10 minutes apart during rest and adenosine stress. Dynamic PET data were acquired in six human subjects using imaging protocols that provided separate single-injection scans as gold standards. Rest and stress data were combined to emulate rapid dual-injection data so that the underlying activity from each injection was known exactly. Regional blood flow estimates were computed from the dual-injection data using two methods: background subtraction and combined modeling. The rapid dual-injection approach provided blood flow estimates very similar to the conventional single-injection standards. Rest blood flow estimates were affected very little by the dual-injection approach, and stress estimates correlated strongly with separate single-injection values ($r = 0.998$; mean absolute difference = 0.06 ml/min/g). An actual rapid dual-injection scan was successfully acquired in one subject and further demonstrates feasibility of the method. This study with a limited dataset demonstrates that blood flow quantification can be obtained in only 20 minutes by the rapid dual-injection approach with accuracy similar to that of conventional separate rest and stress scans. The rapid dual-injection approach merits further development and additional evaluation for potential clinical use.

1. Introduction

Measurements of myocardial blood flow (MBF) at rest and during stress are valuable for detection and assessment of ischemia secondary to coronary artery disease (CAD) and monitoring response to therapeutic interventions (Kaufmann and Camici 2005). Positron emission tomography (PET) can provide non-invasive quantification of perfusion using dynamic imaging and tracer kinetic modeling. One of the best myocardial perfusion tracers in PET is ^{13}N -ammonia (half-life = 9.97 min), which becomes metabolically trapped in myocytes and produces high quality images. Kinetic modeling and blood flow quantification methods for ^{13}N -ammonia are well established and validated (Krivokapich *et al.* 1989; Hutchins *et al.* 1990; Bol *et al.* 1993; Muzik *et al.* 1993; Nitzsche *et al.* 1996; Choi *et al.* 1999). Current methods for performing rest and stress ^{13}N -ammonia cardiac PET require two separate scans with a waiting period of one hour or more between the scans to allow for radioactive decay. This waiting period allows for radioactivity from the first injection to decay to a point where it does not interfere significantly with the later scan.

The objective of this study was to investigate whether or not rest and stress myocardial blood flows can be accurately quantified using a single dynamic PET scan with a short, 10 minute delay between ^{13}N -ammonia injections. The ability to perform both a rest and stress myocardial perfusion study in a shorter period of time using ^{13}N -ammonia would be quite valuable from a clinical standpoint. The proposed rapid dual-injection, single-scan imaging approach is illustrated in figure 1. After the patient is positioned in the scanner and a transmission scan has been acquired for attenuation correction, dynamic PET is performed continuously while injections of ^{13}N -ammonia are administered at the scan start during rest and 10 min later during adenosine stress. This rapid dual-injection approach reduces the overall procedure time significantly compared to conventional single-injection methods. Potential advantages include increased scanner throughput and utilization, improved co-registration of rest and stress data, reduced motion artifact, reduced transmission scan radiation exposure, and improved patient comfort and convenience. Another advantage is that the two doses of ^{13}N -ammonia for rest and stress injections may be obtained from a single cyclotron run and split for the rapid sequential injections.

Quantification of myocardial perfusion using rapid dual-injection single-scan ^{13}N -ammonia PET presents a technical challenge. While rest measurements are unaffected by the dual-injection approach, stress measurements are complicated by significant interference from the rest injection. The background activity from the rest injection remaining in the myocardium during the stress measurement is typically 10–20% of the total activity, and can be even higher in cases with a stress defect. Processes such as tracer kinetics, metabolic trapping, and radioactive decay all need to be carefully considered when applying a kinetic model for quantification of dual-injection data. Though previous studies of rapid dual-injection ^{13}N -ammonia PET are lacking, related work has provided some insight into this problem. Techniques to quantify different physiologic states from PET data with rapid sequential injections of the same tracer have been developed previously for imaging brain metabolism with ^{18}F -fluorodeoxyglucose (FDG) (Chang *et al.* 1987; Chang *et al.* 1989; Nishizawa *et al.* 2001) and various ligand-receptor interactions (Huang *et al.* 1989; Delforge *et al.* 1990). Methods have also been developed to recover individual tracer information from data with overlapping signals from different PET tracers based on different half-lives, tracer kinetics, or both (Huang *et al.* 1982; Koeppe *et al.* 2001; Converse *et al.* 2004; Kadrmas and Rust 2005; Rust and Kadrmas 2006).

In this study, a 20 min dynamic emission scan protocol with ^{13}N -ammonia injections staggered 10 minutes apart was developed and tested using data from six human subjects. Dynamic ^{13}N -ammonia PET data was acquired using an experimental protocol designed to provide “gold standard” rest and stress myocardial blood flow measures along with comparable rapid dual-injection single-scan data. Two straightforward methods, background subtraction and combined modeling, were applied for quantification of regional myocardial blood flows from dual-injection data. Quantitative results from the rapid dual-injection single-scan approach were evaluated versus results from conventional separate single-injection rest and stress scans using linear regression and Bland-Altman analysis (Bland and Altman 1986).

2. Methods

One challenge of evaluating the rapid dual-injection approach in humans is the need for a gold standard. One option would be to acquire separate rest, stress, and dual-injection scans in each subject; however, previous work has shown up to 10–15% variability in global blood flow estimates in repeated scans (Nagamachi *et al.* 1996), and such an approach would require twice the radiation exposure and adenosine effects for each participant. In this work separate rest and stress scans were acquired, providing a standard for each measurement, and the data were combined to emulate a rapid dual-injection acquisition as described in section 2.1.4. In one

subject, an actual rapid dual-injection scan was acquired as an example; separate single-injection scans were not available for comparison in that case.

2.1 Data Acquisition and Processing Methods

2.1.1. Human Subjects—Six human subjects (4 male, 2 female, age 57 ± 9 years, weight 84 ± 19 kg) participated in this study under informed consent and according to an institutionally approved research protocol. Four subjects had low risk of cardiac disease as determined by medical history and physical examination. One subject had received a heart transplant 14 months prior to imaging. Another subject was scanned twice, at two weeks and again at two months after receiving a left ventricular assist device (LVAD) to treat congestive heart failure. These subjects provided seven datasets including two with abnormally low flow regions, so that the rapid dual-injection approach was tested over a wide range of blood flows.

2.1.2. Data Acquisition—All scans were performed using an AdvanceTM PET scanner (GE Medical Systems, Waukesha, WI) operated in 2D mode. Subjects were positioned with arms down and a strap was placed across the chest to immobilize the subject and minimize movement artifacts. Transmission scans (⁶⁸Ge rod source) were acquired to position the heart in the field of view (2 min) and for attenuation correction (10 min). At the start of each emission scan, 21.5 ± 4.0 mCi ¹³N-ammonia was administered intravenously over 20–30 seconds followed by a saline flush. Scans performed during stress were started at the midpoint of a 6 minute adenosine infusion (0.14 mg/kg/min) while vital signs including pulse rate, blood pressure, and oxygen saturation were assessed by a physician using standard monitoring equipment.

Dynamic PET data were acquired with a waiting period of 50–71 minutes between rest and stress emission scans. Imaging protocols were designed to allow rest and stress components to be added to emulate rapid dual-injection data as described in section 2.1.4. Rest and stress emission scan data were acquired separately using the following temporal sampling schedule for the first 10 minutes (12×5 sec, 6×10 sec, 6×30 sec, and 5×1 min). For rest scans, this schedule was repeated for another 10 minutes for a total emission scan duration of 20 minutes. This provided equal duration stress timeframes that could be shifted by in time and added to the rest timeframes in order to mimic a rapid dual-injection acquisition while retaining standard single-injection emission scan sequences. In addition, an actual rapid dual-injection PET scan was acquired in one subject; in that case, one injection of ¹³N-ammonia was given at the scan start during rest, adenosine was infused from 7–13 minutes, and a second tracer injection was given at 10 minutes during stress.

2.1.3. Data Processing—Images were reconstructed using filtered back-projection with a Hanning window cutoff at 1.56 cycles/cm. The 128×128 image matrices had 4.3 mm voxels and a slice thickness of 4.25 mm. Data were pre-corrected for attenuation, scatter, and randoms prior to reconstruction, but not for radioactive decay because it was included in the kinetic modeling equations. A reference image for each scan was created by summing up timeframes with steady uptake of tracer in the myocardium and used as a guide for reorienting to cardiac short-axis (SA) images. The SA images for all rest and stress timeframes in each subject were co-registered using a template showing the endocardial and epicardial boundaries. Based on visual comparison of an average mid-ventricular slice from each timeframe, the entire SA image volume was shifted manually to align with the template in the plane perpendicular to the SA of the heart. Co-registration was not performed in timeframes where myocardium and blood pool were indistinguishable.

Myocardial regions were selected based on the 17 segment model of the American Society of Nuclear Cardiology (Schelbert *et al.* 2003). On each slice of a reference image, endocardial and epicardial boundaries were drawn and the angle of insertion of the right ventricle into the

septum was selected as a landmark for division into sectors. The myocardium was divided into: 1 apical region, 4 sectors in the apical mid-ventricle, 6 sectors in the basal mid-ventricle, and 6 sectors in the base of the heart. Blood pool regions were drawn manually in each subject with size restricted to limit partial volume effect and spillover of activity from the myocardium. In each subject, the same regions were applied to all co-registered rest and stress timeframes. Time activity curves were computed as the average activity concentration in the region at the midpoint of each timeframe, and activity was set to zero prior to the initial bolus entering the blood pool.

2.1.4. Emulation of Rapid Dual-Injection Data—Separate single-injection rest and stress scans were acquired as gold standards, and the data from these scans were also combined to emulate rapid dual-injection imaging. This approach provided dual-injection data where the components of activity from the rest and stress injections were known exactly. Regional myocardial blood flow estimates were computed from the dual-injection data and evaluated versus estimates obtained from the separate rest and stress components. This approach eliminated factors that usually contribute to differences in blood flow measures in repeated scans, including differences in noise realizations, region of interest selection, patient movement, and physiological changes. Interference due to signal overlap and the methods used to quantify blood flow were the only sources of differences between the rapid dual-injection results and separate single-injection standards. A possible limitation of this approach is that the component of activity from the rest injection was not affected by adenosine, which would be infused from 7–13 minutes during an actual rapid dual-injection scan and could affect the tail end of the rest component (see figure 1).

Rapid dual-injection dynamic PET data were emulated by combining the rest and stress time-activity curves for each myocardial region after the data were reconstructed and images co-registered. Rest and stress data were acquired separately using the dynamic sampling schedules described in section 2.1.2. The stress time-activity curves were shifted in time and added to the rest curves to mimic a 20 minute rapid dual-injection acquisition with a 10 minute delay between injections. The 10 minute delay was chosen to provide a good tradeoff between imaging time and quantitative accuracy based on preliminary simulations and previous work with a similar tracer (Rust and Kadrmas 2006). This delay provides enough time to obtain estimates of rest blood flow without interference from the stress injection, and allows tracer kinetics from the rest injection to stabilize before the start of adenosine infusion.

2.2. Quantification Methods for Rapid Dual-Injection Data

2.2.1 Compartment Model—Quantitative estimates of myocardial blood flow were obtained by fitting the compartment model shown in figure 2 to the dynamic PET data (Hutchins *et al.* 1990; Choi *et al.* 1999). Using this model, the ^{13}N activity concentration in a myocardial region-of-interest, $R(t)$, can be written:

$$R(t) = f_B B(t) + (1 - f_B) A(t), \quad [1]$$

where f_B is the total fractional blood volume, $B(t)$ is the ^{13}N activity concentration in the whole blood, and $A(t)$ is the ^{13}N activity concentration in the myocardial tissue. For conventional separate single-injection rest and stress scans, $A(t)$ can be modeled including the effects of radioactive decay as:

$$A(t) = \left[\frac{k_1 k_3}{k_2 + k_3} e^{-\lambda t} + \frac{k_1 k_2}{k_2 + k_3} e^{-(k_2 + k_3 + \lambda)t} \right] \otimes b(t), \quad [2]$$

where $\{k_i\}$ are kinetic rate constants, λ is the radioactive decay constant, $\otimes$ is the convolution operator, and $b(t)$ is the metabolite-corrected blood input function. The input function, $b(t)$, is the fraction of un-metabolized and freely exchangeable ^{13}N -ammonia available in the whole blood, $B(t)$, at time t . In this study, metabolite correction was performed based on average values reported in the literature (Rosenspire *et al.* 1990; Bormans *et al.* 1995). A modified Levenberg-Marquardt algorithm for chi-squared minimization was used to find the best fit parameters (Press *et al.* 1992), where the data were weighted by the timeframe durations in order to compensate for nonuniform temporal sampling. The kinetic rate constants were limited to non-negative values, k_2 was constrained to be less than or equal to k_1 , and f_B was limited to range from zero to one. Using this model, k_1 provided a quantitative estimate of myocardial perfusion with units of ml/min/g.

For the separate single-injection scans only the first 10 minutes of data were used and fit to the model, and for the rapid dual-injection scans the full 20 minutes of dynamic data were used as described in the following sections. Rapid dual-injection data could be represented using an equation of the same general form as equation [1]; however, kinetic parameters would not be constant due to the infusion of adenosine during the scan. Development of an advanced kinetic model that exactly matched actual rapid dual-injection data including transient changes in blood flow due to adenosine infusion was not attempted, and would not be applicable to the emulated dual-injection data used to obtain our gold standard. The basic model applied in this work considers the dual-injection data to be the sum of time-activity curve components from rest and stress injections, where each component is represented using the model described above. With this limitation, two straightforward methods for blood flow quantification were applied to test the rapid dual-injection approach as described below. Separate rest and stress whole blood curves and metabolite-corrected input functions were needed to apply these methods and were obtained from the dual-injection data as described below in section 2.2.4.

2.2.2 Background Subtraction Method—One straightforward method for quantification of rapid dual-injection data is to treat the stress data analysis as a background subtraction problem. This method considers the data as the sum of activity from two injections. The components from each injection are separated in several stages: 1) a kinetic model is fit to the early phase of the time-activity curve when only activity from the first injection is present; 2) an estimate of the radioactivity from the first injection during the late phase is obtained by extrapolation; 3) the extrapolated component from the first injection is subtracted from the total activity during the late phase to recover an estimate of the radioactivity from the second injection. Once the time-activity curves for each injection are separated, standard quantification methods can be applied to each component. One potential problem with this method is that the extrapolated estimate of activity from the first injection may be inaccurate because it is based on only 10 minutes of data and doesn't account for later changes due to adenosine.

In this work, the conventional model described above was fit to the first 10 minutes of dual-injection data to estimate kinetic parameters during the rest part of the scan. As a result, the rest blood flow estimates were identical to what would be obtained from a separate single-injection rest scan. For the background subtraction method, the rest kinetic parameters were used to extrapolate the ^{13}N -activity concentration in the heart for the stress portion of the scan over the period from 10–20 minutes:

$$R_{\text{Ext}}^{\text{Rest}}(t > 10 \text{ min}) = f_B B^{\text{Rest}}(t) + (1 - f_B) A^{\text{Rest}}(t), \quad [3]$$

where $B^{\text{Rest}}(t)$ is the rest whole blood curve obtained from the dual-injection data as described in section 2.2.4, and $A^{\text{Rest}}(t)$ is the extrapolated activity concentration in the tissue from the

rest injection. Stress kinetic parameters were estimated by fitting the recovered stress curve, which was obtained by subtracting the extrapolated rest curve from the dual-injection data:

$$\left[R^{\text{Dual}}(t) - R_{\text{Ext.}}^{\text{Rest}}(t) \right] = f_B B^{\text{Stress}}(t) + (1 - f_B) A^{\text{Stress}}(t), \quad [4]$$

where f_B and $\{k_i^{\text{Stress}}\}$ are estimated by the fitting procedure. The separate stress whole blood curve, $B^{\text{Stress}}(t)$, and input function were obtained from the dual-injection data as described in section 2.2.4.

2.2.3. Combined Modeling—The application of a combined model, which can account for the activity from both injections and the two different physiologic states, was also tested in this work. Using this method, kinetic parameters for rest and stress were simultaneously estimated by performing a fit to all of the rapid dual-injection data. Similar methods have been developed and investigated in previous studies (Huang *et al.* 1989; Delforge *et al.* 1990; Koeppe *et al.* 2001; Kadrmaz and Rust 2005; Rust and Kadrmaz 2006). The combined modeling method allows greater flexibility compared to the background subtraction method in terms of adapting the model configuration to match tracer kinetics during an actual rapid dual-injection scan. A potential disadvantage of combined modeling is that the parameter estimation procedure is more computationally challenging.

In this work, a combined model applicable to the emulated data was tested in order to determine whether or not all the rest and stress parameters could be simultaneously estimated from the rapid dual-injection data. The total ^{13}N activity concentration in a myocardial region, $R^{\text{Dual}}(t)$, can be written as the sum of time-activity curves for rest and stress injections:

$$R^{\text{Dual}}(t) = \left[f_B^{\text{Rest}} B^{\text{Rest}}(t) + (1 - f_B^{\text{Rest}}) A^{\text{Rest}}(t) \right] + \left[f_B^{\text{Stress}} B^{\text{Stress}}(t) + (1 - f_B^{\text{Stress}}) A^{\text{Stress}}(t) \right], \quad [5]$$

where f_B^{Rest} , $\{k_i^{\text{Rest}}\}$, f_B^{Stress} , and $\{k_i^{\text{Stress}}\}$ are simultaneously estimated by the fitting procedure. This model configuration was designed to match the emulated dual-injection data in this study, which were formed by combining separate rest and stress scans. Some potential modifications that may improve the combined model for actual dual-injection scans are presented in the discussion section. The combined model was fit to all 20 minutes of dual-injection data, and a total of eight parameters were recovered by the fitting procedure. Fits to the dual-injection data were performed using the same algorithm, parameter constraints, initial values, and number of iterations as used in the fits to separate single-injection data. Several different weighting strategies were investigated in this work, all of which provided similar results. This combined model was also used to fit the data from the actual rapid dual-injection scan acquired in one person as an example.

2.2.4. Blood Curve Separation—Separate rest and stress blood curves need to be recovered from the dual-injection data in order to quantify blood flow using the methods described above. These curves are not readily available from the images because there is a small amount of residual ^{13}N activity from the rest injection in the blood pool region that overlaps with the activity from the stress injection during the period from 10–20 minutes (see figure 3). However, previous studies of ^{13}N -ammonia metabolism in the blood have shown that a large fraction of the total residual ^{13}N activity in the blood is metabolically trapped within 10 minutes post-injection (Rosenspire *et al.* 1990; Bormans *et al.* 1995). Twenty minutes of separately acquired rest data were available in this study and used to test several models for extrapolating the rest whole blood curve for dual-injection data. Very little difference was

observed between the various models, and we found that the ^{13}N activity concentration in whole blood from the rest injection, $B^{\text{Rest}}(t)$, could be modeled simply and reliably as a constant times radioactive decay during the period from 10–20 minutes:

$$B^{\text{Rest}}(t) = \alpha e^{-\lambda t}, \quad [6]$$

where α is unknown, and λ is the radioactive decay constant. For dual-injection data, α was obtained by performing a least-squares fit to the blood pool time-activity curve using timeframes from 5–10 minutes post-injection. The residual ^{13}N activity in the whole blood from the rest injection was then extrapolated over the period from 10–20 minutes and subtracted from the dual-injection blood curve to obtain the stress whole blood curve, $B^{\text{Stress}}(t)$. Input functions for each component were obtained by correcting the whole blood curves for ^{13}N -labeled metabolites starting from the time of each injection and based on average values reported in the literature (Rosenspire *et al.* 1990; Bormans *et al.* 1995).

The measured rest and stress blood curves were available in this study because the dual-injection data was emulated by adding separately acquired rest and stress components, and the effects of the blood curve separation procedure were assessed using these measured curves. The mean difference ($\pm$ standard deviation) between blood flow estimates obtained from dual-injection data using the separation procedure compared to using the measured rest and stress blood curves was 0.016 ± 0.023 ml/min/g.

3. Results

High quality dynamic PET imaging data was successfully obtained for each of the six human subjects, providing a wide range of blood flows. Regional myocardial blood flow estimates were computed in 17 regions per subject ($n=102$), with 1 region in the LVAD patient where the fitting routine failed to provide reasonable results. The mean $\pm$ standard deviation of myocardial blood flow estimates over 101 myocardial regions based on the separate single-injection results in these subjects was 0.70 ± 0.36 ml/min/g at rest and 2.25 ± 1.70 ml/min/g during stress. Four of the subjects provided blood flow values in a normal range (rest: 0.91 ± 0.23 ml/min/g, stress: 3.20 ± 1.24 ml/min/g), and the subject with a left ventricular assist device provided two datasets with abnormally low flows at rest (0.28 ± 0.13 ml/min/g) and during stress (0.31 ± 0.11 ml/min/g).

3.1. Single Region Example

Rapid dual-injection data for a typical region is shown in figure 3, along with a fitted curve obtained using the combined modeling method. Quantitative results for this case are provided in table 1, where the uncertainties are estimates of standard deviations obtained from the formal covariance matrix of the fit on the assumption of normally distributed errors (Press *et al.* 1992). In the first 1–2 minutes following each injection, there was a distinct increase in the dual-injection time-activity curve. This increase included a sharp peak arising from the blood component ($f_B \approx 0.5$) and an elevated plateau due to ^{13}N uptake by the myocardial tissue. By 10 minutes, the activity from the rest injection was mostly stable except for radioactive decay, and the additional uptake from the stress injection was clearly distinguishable. The results in table 1 show that the rapid dual-injection approach had only a small effect on blood flow quantification. Note that rest parameter estimates obtained from dual-injection data using the background subtraction method were identical to separate single-injection estimates because both were obtained using only the first 10 minutes of data, and hence were not affected by using the rapid dual-injection protocol. For the case shown in figure 3, the stress MBF estimate from background subtraction was slightly higher than the single-injection value, differing by about 5%. Using the combined modeling method, rest and stress parameters were recovered

simultaneously by fitting all 20 minutes of dual-injection data. Rest and stress MBF estimates from combined modeling were both within 4% of the separate single-injection estimates for this region. As shown in figure 3, the fitted curve from combined modeling closely matched the dual-injection data.

3.2. Rapid Dual-Injection vs. Separate Single-Injection Method

The rapid dual-injection approach provided estimates of myocardial blood flow both for rest and stress very similar to the conventional separate single-injection standards. Results are first presented for the background subtraction method of recovering rest and stress MBF values from the dual-injection data, and results for the more involved combined modeling method will be presented in section 3.3. Recall that for background subtraction, rest parameter estimates were obtained using the first 10 minutes of data and were identical to those obtained from separate single-injection data. An estimate of the rest activity was then removed from the dual-injection data to recover the stress component. Background subtraction recovered stress blood flow values from dual-injection data very similar to the separate single-injection results over the entire range of values.

Figure 4 provides a direct comparison of the stress MBF estimates for the background subtraction method versus separate single-injection results for each myocardial region. Background subtraction and the standard single-injection approach provided similar values in all cases, and regions with abnormally low flow were clearly delineated from high flow regions by both methods. Regression analysis was performed to compare MBF estimates from background subtraction versus the standards. As shown by the equation in figure 4, a very strong correlation was observed ($r = 0.998$, slope = 1.025, and intercept = 0.023). Close examination of the small differences in MBF estimates revealed a slight tendency towards overestimation by the background subtraction method compared to the separate single-injection method at normal to high blood flow levels. Overall, these results suggest that the rapid dual-injection approach can provide blood flow estimates very similar to conventional separate single-injection rest and stress scans.

3.3. Simultaneous Rest and Stress Quantification by Combined Modeling

The combined modeling method was investigated in this work to determine whether or not blood flow estimates for rest and stress could be simultaneously recovered from dual-injection data. This section builds on and support previous rapid dual-tracer PET studies where similar kinetic modeling methods have been applied. Compared to background subtraction, the combined modeling method is more adaptable to match the tracer kinetics expected during actual dual-injection scans. Rest and stress parameters for each myocardial region were recovered by fitting the combined model to 20 minutes of dual-injection data. Rest and stress MBF estimates from combined modeling were very similar to the separate single-injection gold standards. Figure 5 compares blood flow estimates obtained from rapid dual-injection data using combined modeling versus the separate single-injection standards. The small differences in values obtained from these two methods are examined more closely in figure 6.

3.3.1. Rest Results—Figures 5(a) and 6(a) demonstrate that fitting the combined model to the dual-injection data provided rest perfusion estimates very similar to the single-injection standards. Although rest estimates identical to the conventional (single-injection) values could have been obtained by performing a separate fit to the first 10 minutes of data, the combined modeling method provided nearly identical results in a single step. As shown in figure 5(a), the correlation between rest MBF estimates from combined modeling versus standard single-injection values was very strong ($r > 0.999$). In figure 6(a), The differences between rest MBF estimates from combined modeling and standard values for each region are plotted versus the average value from the two methods. This Bland-Altman plot shows that the differences

between the two methods were consistently small across the entire range of resting blood flow values in this study. These results show that simultaneous estimation of rest and stress parameters does not have a significant effect on rest results.

3.3.2 Stress Results—Figures 5(b) and 6(b) show that combined modeling provided very similar stress blood flow estimates as compared to the separate single-injection method. Combined modeling also provided similar performance as compared to the background subtraction method. Figure 5(b) directly compares stress MBF estimates from the combined modeling method versus single-injection standards for each region. As shown on the plot, the correlation between MBF estimates from combined modeling versus the standards was very strong ($r = 0.998$, slope = 1.016, intercept = 0.022). Strong correlations versus single-injection results were also observed for f_B ($r = 0.990$) and k_2 ($r = 0.996$), with somewhat greater variability for k_3 estimates ($r = 0.953$).

The Bland-Altman plot in figure 6(b) allows closer examination of the small differences between stress MBF estimates from combined modeling and the separate single-injection method for each region. Low flow regions were clearly delineated from high flow regions in these data using the rapid dual-injection approach. The mean $\pm$ standard deviation of the MBF differences was 0.06 ± 0.11 ml/min/g, and these differences are small relative to the magnitude of clinically-significant flow variations. By comparison, the background subtraction method resulted in a slightly higher mean difference versus single-injection values, 0.08 ± 0.12 ml/min/g. Based on the separate single-injection values, the rapid dual-injection approach tended to slightly overestimate stress MBF (approximately 3%) at normal to high blood flows. This subtle effect may be due to the weights used to perform the fits, or because the noise in the reconstructed images is not exactly Gaussian.

The combined modeling method can also be applied on a voxel-by-voxel basis to recover separate rest and stress images. Using the results of the fit, the relative fraction of activity from the rest and stress injections in each timeframe can be predicted. This data can then be used to separate dual-injection data into rest and stress components. This procedure was applied to the dynamic images, and the results were summed from 5–10 minutes to produce static images. Figure 7 shows a conventional short-axis stress image, the dual-injection image containing both rest and stress components, and the recovered stress image. The recovered image closely matched the single-injection image, as shown by the profiles in the figure. Further work would be necessary to assess the diagnostic performance of stress images recovered in this manner.

3.4. Actual Rapid Dual-Injection Scan

An actual rapid dual-injection scan was acquired in one subject in order to demonstrate the feasibility of this approach and to provide an example dataset. Short-axis images of the myocardium and time-activity curves for the blood pool and a typical region are shown in figure 8. A 20 min dynamic emission scan was acquired with 18.9 mCi of ^{13}N -ammonia injected at the scan start during rest, adenosine infused from 7–13 min, and 18.7 mCi of ^{13}N -ammonia injected at 10 min during stress. Notably, both injected doses of ^{13}N -ammonia were obtained from a single cyclotron run. Images were reconstructed and processed using methods similar to those described in section 2.1.3. Regional myocardial blood flows were quantified using the combined modeling method described in section 2.2.3 and the blood curve separation procedure described in section 2.2.4. As shown in figure 8, high quality images were obtained by summing timeframes from 5–10 minutes at rest and by summing timeframes from 15–20 minutes at stress. The mean $\pm$ standard deviation of MBF estimates over the 17 myocardial regions in this subject were 0.69 ± 0.10 ml/min/g at rest and 2.63 ± 0.51 ml/min/g during stress, and perfusion flow reserves (stress/rest) were 3.96 ± 1.06 . Qualitatively, the data for this subject

appeared very similar to the emulated dual-injection data used in this study, and the activity from the rest injection was not significantly affected by adenosine.

4. Discussion

The rapid dual-injection approach was tested in this work using emulated data from human subjects which offered several advantages and limitations. As described in section 2.1.4, using emulated data allowed the effects of rapid dual-injection imaging to be examined independently of other factors that contribute to variations in blood flow estimates. This experiment was designed to test whether or not the rapid dual-injection approach could provide regional blood flow estimates similar to conventional separate single-injection imaging. Additional studies with an independent gold standard, such as radiolabeled microspheres in an animal model, would be needed to directly evaluate the accuracy of the rapid dual-injection approach versus conventional single-injection imaging. However, the results of this study demonstrate that the differences between the rapid dual- and separate single-tracer methods are smaller than variations in blood flow estimates due to statistical noise, region of interest selection, patient motion, and physiological changes (Nagamachi *et al.* 1996), so making such a comparison may be unwarranted. The main limitations of using emulated dual-injection data were that the rest injection activity was not affected by adenosine stress at 7 minutes post-injection, and that deadtime and randoms were slightly smaller than would be encountered in an actual dual-injection scan. For an actual rapid dual-injection scan, there could be some residual exchangeable tracer leftover from the rest injection at the time that adenosine is infused. This could then lead to some additional uptake of the rest activity into the myocardium. Although the magnitude of this effect appears to be small based on figures 3 and 8, additional work is needed to determine its importance and to adapt the method if necessary. Another consideration is the possibility of shape distortion in the myocardium due to ischemic changes during stress that may require techniques such as image warping to align the rest and stress images.

The model configuration used in this work was designed to match the emulated data, and does not exactly match actual rapid dual-injection data. Development of a combined model that more accurately describes the tracer kinetics that occur during rapid dual-injection scans may potentially improve the accuracy of this approach. For example, in equation [5] there are two copies of f_B and two different sets of kinetic rate constants in play at any point in time, which is not physiologically realistic. One alternative would be to include in the modeling equations an instantaneous change or gradual transition from rest to stress parameters to account for physiological changes, as in (Chang *et al.* 1987). However, our preliminary dual-injection scan suggests that starting the adenosine infusion 7 minutes after the rest injection has little effect upon the rest component, in which case the quantitative methods proposed in this work may be suitable for actual rapid dual-injection data.

The rapid dual-injection approach can provide rest and stress blood flow estimates with a total emission scan time of 20 minutes. In this work, the same injected dose was used for both the rest and stress injections; however, it may be possible to improve rapid dual-injection performance by using a somewhat higher dose for the stress injection (as studied in Rust and Kadrmas 2006). While it could be possible to further reduce the delay between rest and stress injections, this would increase interference between the rest and stress components, potentially reducing quantitative accuracy. Furthermore, it's unlikely that this would significantly shorten the total procedure time when considering the time required for patient positioning and transmission scanning. Using the rapid dual-injection approach, rest and stress data can be obtained with total procedure times similar to those for short half-life blood flow tracers, such as ^{15}O -water (half-life = 122 sec) and ^{82}Rb (half-life = 76 sec), while bringing benefits such as higher counts for gated studies. Previous studies have shown that ^{13}N -ammonia and ^{15}O -water have advantages for quantification of blood flow as compared to ^{82}Rb , which is limited

by the heavy dependence of myocardial extraction on the flow rate and metabolic state (Kaufmann and Camici 2005). Also, since ^{13}N -ammonia is a trapped tracer it can provide images that clearly delineate the myocardium as compared to ^{15}O -water which is freely diffusible.

5. Summary and Conclusions

In this study, a rapid dual-injection single-scan approach for quantification of rest and stress cardiac blood flows using dynamic ^{13}N -ammonia PET was proposed and tested using data from human subjects. Separate single-injection rest and stress scans were acquired in order to obtain a standard for evaluation of dual-injection blood flow estimates. The rest and stress data were combined to emulate 20 minute long rapid dual-injection scans with injections staggered 10 minutes apart, and an actual rapid dual-injection scan was acquired in one subject to further demonstrate the feasibility of this approach. Quantitative blood flow estimates were computed from the dual-injection data using two methods: background subtraction and combined modeling. Use of the rapid dual-injection approach had little effect upon rest blood flow estimates. Stress blood flow estimates were very similar to the separate single-injection standards ($r = 0.998$; mean absolute difference = 0.06 ml/min/g), and low flow regions were clearly delineated from high flow regions. The combined modeling method succeeded at simultaneously estimating both rest and stress blood flow estimates in a single step, with an accuracy slightly better than the background subtraction method. This study with a limited dataset demonstrates that blood flow quantification can be obtained in only 20 minutes by the rapid dual-injection approach with accuracy similar to that of conventional separate rest and stress scans. Additional work is needed to refine the methods and evaluate this approach in a larger clinical population before routine application should be considered.

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(a) Conventional Separate Single-Injection Scans

(b) Rapid Dual-Injection Single-Scan Approach

Figure 1.

Conventional methods for measuring cardiac blood flow at rest and during stress with ^{13}N -ammonia PET require a waiting period of about an hour between scans to allow for radioactive decay, as shown in (a). In the proposed rapid dual-injection approach shown in (b), ^{13}N -ammonia is injected at the scan start at rest and again 10 min later during adenosine stress during a single continuous dynamic emission scan. Quantification methods for dual-injection data must account for the change in physiologic states and activity from each tracer injection.

Figure 2.

Compartment model used to quantify blood flow from rapid dual-injection PET data. A two tissue-compartment model with irreversible trapping was applied for rest and stress with two different sets of rate parameters. Separate blood input functions for the rest and stress injections were obtained from the dual-injection data, and the effects of radioactive decay were included in the modeling equations.

Figure 3.

Typical example of emulated rapid dual-injection data used in this study. Time-activity curves (which have not been decay corrected) are shown for the blood pool and one myocardial region, along with a fitted curve obtained using the combined modeling method. Increases in the activity concentration are clearly distinguishable following the rest and stress injections, and the activity stabilizes within a few minutes after each injection. The rest and stress input functions were recovered from the whole blood curve as described in section 2.2.4. and quantitative results for this case are shown in Table 1.

Figure 4.

Scatter plot of stress blood flow estimates ($n = 101$ regions, 6 subjects) obtained from rapid dual-injection data using the background subtraction method versus values from conventional single-injection imaging. The blood flow estimates from the rapid dual-injection method correlated very strongly with the separate single-injection standards, and both methods clearly delineated the low and high flow regions.

Figure 5.

Scatter plots of rest (a) and stress (b) blood flow estimates ($n = 101$ regions) obtained from rapid dual-injection data using the combined modeling method versus standard single-injection values. In the combined modeling method, kinetic parameters for rest and stress were simultaneously estimated by fitting all 20 min of dual-injection data. Both the rest and stress blood flow estimates correlated very strongly with the conventional single-injection standards, as reflected by the regression lines and correlation coefficients listed on the plots.

Figure 6.

Differences in blood flow estimates obtained from dual-injection data using the combined modeling method versus separate single-injection estimates are plotted versus the average values for rest (a) and stress (b). The mean difference ± 2 standard deviations are shown on the stress plot. These results indicate that the rapid dual-injection approach had very little affect on rest blood flow estimates and only a small affect on stress blood flow estimates.

Figure 7.

Example of recovering the static short-axis stress image from rapid dual-injection data. Top row, left to right: conventional single-injection stress image, rapid dual-injection image with activity from both rest and stress, and recovered stress image. Bottom row: profiles demonstrating that the recovered stress image closely matched the single-injection standard. Stress images were recovered by performing combined modeling fits on a voxel-by-voxel basis, and using the fit results to estimate the fraction of activity from the rest and stress injections in each timeframe.

Figure 8.

Example dynamic data from the actual rapid dual-injection scan. Short-axis images summed over the timeframes from 5–10 minutes and 15–20 minutes are shown on top, and time-activity curves for the blood pool and a typical myocardial region, along with the fitted curve obtained using combined modeling are shown below. The activity from the rest injection did not appear to be significantly affected by adenosine, which was infused from 7–13 min during the scan. The mean $\pm$ standard deviation of blood flow estimates over 17 regions was 0.69 ± 0.10 ml/min/g at rest and 2.63 ± 0.51 ml/min/g during stress using the combined modeling method.

Table 1Quantitative results for the example case^a.

Parameter	Separate Single-Injection	Background Subtraction	Combined Modeling
k_1^{Rest}	0.92 ± 0.05^b	0.92 ± 0.05	0.93 ± 0.05
k_2^{Rest}	0.13 ± 0.04	0.13 ± 0.04	0.14 ± 0.04
k_3^{Rest}	0.08 ± 0.07	0.08 ± 0.07	0.11 ± 0.06
f_B^{Rest}	0.47 ± 0.02	0.47 ± 0.02	0.47 ± 0.02
k_1^{Stress}	3.33 ± 0.27	3.50 ± 0.32	3.44 ± 0.32
k_2^{Stress}	0.69 ± 0.12	0.80 ± 0.17	0.79 ± 0.17
k_3^{Stress}	0.20 ± 0.03	0.24 ± 0.04	0.24 ± 0.04
f_B^{Stress}	0.58 ± 0.04	0.56 ± 0.05	0.55 ± 0.05

^aTime-activity curves for this case are shown in figure 3.^bStandard deviations estimated from formal covariance matrix.