Optimal Adenosine Stress for Maximum Stress Perfusion, Coronary Flow Reserve, and Pixel Distribution of Coronary Flow Capacity by Kolmogorov–Smirnov Analysis

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Background—Different adenosine stress imaging protocols have not been systemically validated for absolute myocardial perfusion and coronary flow reserve (CFR) by positron emission tomography, where submaximal stress precludes assessing physiological severity of coronary artery disease.

Methods and Results—In 127 volunteers, serial rest–stress positron emission tomography scans using rubidium-82 with various adenosine infusion protocols identified (1) the protocol with maximum stress perfusion and CFR, (2) test–retest precision in same subject, (3) stress perfusion and CFR after adenosine compared with dipyridamole, (4) heterogeneity of coronary flow capacity combining stress perfusion and CFR, and (5) potential relevance for patients with risk factors or coronary artery disease. The adenosine 6-minute infusion with rubidium-82 injection at 3 minutes caused CFR that was significantly 15.7% higher than the 4-minute adenosine infusion with rubidium-82 injection at 2 minutes and significantly more homogeneous by Kolmogorov–Smirnov analysis for histograms of 1344 pixel range of perfusion in paired positron emission tomographies. In a coronary artery disease cohort separate from volunteers of this study, compared with the 3/6-minute protocol, the 2/4-minute adenosine protocol would potentially have changed 332 of 1732 (19%) positron emission tomographies at low-risk physiological severity CFR ≥2.3 to CFR <2.0, thereby implying high-risk quantitative severity potentially appropriate for interventions but because of suboptimal stress of the 2/4 protocol in some patients.

Conclusions—The 6-minute adenosine infusion with rubidium-82 activation at 3 minutes produced CFR that averaged 15.7% higher than that in the 2/4-minute protocol, thereby potentially providing essential information for personalized management in some patients. **(*Circ Cardiovasc Imaging*. 2017;10:e005650. DOI: 10.1161/CIRCIMAGING.116.005650.)**

Key Words: adenosine ■ adenosine stress ■ cardiac positron emission tomography ■ coronary flow ■ coronary flow reserve ■ Kolmogorov–Smirnov statistic ■ quantitative myocardial perfusion

Cardiac positron emission tomography (PET) offers physiological, noninvasive, quantitative, regional, and global absolute myocardial perfusion in cubic centimeters (cc)/min/g and coronary flow reserve (CFR) for presence and physiological severity of focal stenosis and diffuse coronary artery disease (CAD) to guide its management.[1–4]

See Editorial by Knaapen
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The adenosine stress protocol recommended by the American College of Cardiology/American Heart Association and American Society of Nuclear Cardiology includes 2 protocols: (1) 6-minute infusion of adenosine 140 μg/kg per minute with radionuclide injection at 3 minutes and (2) 4-minute infusion of adenosine 140 μg/kg per minute with radionuclide injection at 2 minutes.[1,2] However, to our knowledge, these protocols have not been systemically validated for obtaining maximum absolute myocardial perfusion, CFR, or stress perfusion heterogeneity by comparative severity distribution histograms, where submaximal stress precludes accurate assessment of physiological severity.

Randomized trials of fractional flow reserve (FFR)–guided percutaneous coronary interventions established the first objective, quantitative, outcomes-driven standard of physiological stenosis severity for guiding revascularization.[5–8] However, pressure-derived FFR requires invasive coronary angiogram and indicates only relative CFR.[3,9] Moreover, FFR was originally validated by comparison to CFR by quantitative PET.[9] With increasing role and importance of noninvasive quantitative myocardial perfusion for guiding management

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of CAD, achieving maximal hyperemia is crucial for accurately quantifying physiological severity. For these reasons, our recent report on regadenoson systematically showed substantially higher quantitative stress perfusion with a modification of the standard regadenoson protocol.[10] Accordingly, we systematically investigated the optimal adenosine infusion protocol for achieving maximal myocardial perfusion by quantification PET.

Methods

From September 2015 to May 2016, we recruited volunteers aged ≥40 years for 2 serial PET perfusion studies within 3 weeks, but at least 1 day apart for this adenosine study. Separately, in our clinical database, 1732 PET scans were done on patients with documented CAD by angiogram, coronary events, revascularization procedures, or ischemia on stress PET (regional stress defect outside 3 SD of healthy normals, electrocardiogram ST segment depression >1 mm, or definite angina requiring aminophylline) undergoing PET for second opinion for procedures, complex coronary anatomy, severity assessment for revascularization, follow up, or unresolved symptoms. Finally, 100 young healthy volunteers aged <40 years with no risk factors and no medical conditions underwent 2 serial standard dipyridamole stress PET on different days for reproducibility of quantitative PET perfusion as an additional reproducibility control for the adenosine study.

All subjects signed informed consent approved by the University of Texas Committee for the Protection of Human Subjects. Subjects were instructed to refrain from caffeine or cigarettes for 24 hours before PET.

Cardiac PET Acquisition and Analysis

Our imaging protocol has been described previously.[3,4,10–18] Cardiac PET was performed using a Discovery ST 16-slice PET–computed tomography scanner (GE Healthcare, Waukesha, WI) in 2-dimensional mode with settings for an in-plane resolution of ≈6 to 7 mm full-width at half-maximum.

Rest emission images were obtained over 7 minutes beginning immediately on intravenous injection of 30 to 50 mCi of generator-produced rubidium (Rb)-82 (Bracco Diagnostics, Princeton, NJ). The first 2-minute emission image provided integrated time–activity arterial input. The last 5-minute emission image provided myocardial uptake image. Depending on the assigned protocol, subjects then underwent stress emission images using adenosine or dipyridamole infusion as pharmacological stress agent and the same dose of radiotracer. 12-lead ECG, heart rate, and blood pressure were recorded.

Low-dose computed tomography scans for attenuation correction were acquired before rest and after stress emission imaging as previously reported.[12] Fusion images superimposed PET emission and computed tomography transmission scans in horizontal, coronal, and sagittal views–optimized coregistration.[12]

PET images were reconstructed using filtered back-projection with postprocessing by tenth-order Butterworth filter (cutoff 15.2 mm). After attenuation correction and reconstruction, transaxial PET images were exported for analysis on HeartSee software (FDA K143664 University of Texas, Houston) to generate true short- and long-axis views, perpendicular and parallel to long axis of left ventricle (LV). Circumferential profiles of maximum radial activity for each true short-axis slice were used to construct 2-dimensional topographical views of LV.

For each radial segment of every short-axis slice, our experimentally validated flow model[13] was implemented in HeartSee software. Our flow model has also been tested by others who reported it to have higher sensitivity for detection and localization of abnormal flow than time–activity curve models.[14] Rest and stress perfusion in cc/min/g and CFR as stress/rest ratio was determined for each of 1344 pixels in the LV image. We use the term CFR instead of myocardial flow reserve to emphasize the general physiological principle independent of measurement technique.

Arterial inputs were personalized for each individual PET from among aortic and left atrium locations because a fixed uniform left atrium or aortic region of interest produces suboptimal arterial input in half of the cases as previously detailed.[15] For most reliable measurement of adenosine dose effects, we used whole heart global stress perfusion and CFR calculated as the average of the 1344 pixels in each LV image. For pixel distribution of stress perfusion and CFR within the 1344 pixels for serial PETs using different stress protocols, we compared histogram distributions in the coronary flow capacity (CFC) map accounting for stress perfusion and CFR of each pixel regionally projected back into that pixel location in LV as previously reported.[3,4,10–17]

Adenosine and Dipyridamole Administration Protocols

Adenosine was infused at 140 μg/kg per minute through an antecubital vein using a single intravenous access 20 or 22-gauge needle sheath with a Y connector (BD Nexiva; 22GA 1.0, N 0.9×25 mm closed IV catheter system with dual port). The saline tubing was connected to a low-pressurized saline bag with a 3-way stopcock to allow choice of simultaneous infusion of saline and adenosine or simultaneous adenosine infusion with Rb-82 slow bolus injection over 15 to 30 seconds followed by turning the stopcock to saline bag to flush through Rb-82. To determine the optimal protocol for quantifying myocardial perfusion by PET, adenosine was administered using various timing protocols shown in Figure 1, protocols A–C. The Rb-82 generator was activated at 2, 3, or 4 minutes after adenosine infusion depending on protocols. Within 3 weeks, subjects returned for another cardiac PET rest/stress scan with different adenosine infusion protocol depending on their randomization of timing sequences.

We evaluated test–retest precision of PET by assigning a group of subjects to the same adenosine infusion protocol for both PET 1 and PET 2 (protocols D and E in Figure 1). As an additional control comparison group, a subset of patients had serial PET, with adenosine and dipyridamole stress done in randomized sequence in the same patient (Figure 2). Dipyridamole (0.56 mg/kg) was infused over 4 minutes (0.142 mg/kg per minute), and Rb-82 generator was activated at 4 minutes after completion of infusion. The radiotracer delivery and PET scanner imaging remained the same in all cases. There was no significant difference in reported symptoms or preference for either adenosine or dipyridamole separate from their sequence.

Statistical Analysis

All data are presented as mean±standard deviation for continuous variables, median and interquartile range for skewed continuous variables, or number and percentage for categorical variables. Paired *t* tests were used to compare for continuous variable, where indicated. Statistical significance was defined as 2-tailed $P<0.05$ for all tests. Coefficient of variance was used to evaluate variability between 2 measurements calculated as coefficient of variance = standard deviation of the difference between 2 measurements/mean value of the 2 measurements.

For comparing histogram distribution of percent of LV in color-coded ranges of CFC integrating stress perfusion with CFR between groups, we used the Kolmogorov–Smirnov (KS) test for differences in histogram distribution.[18] *P* values for the KS statistic were adjusted initially for each pairs of scans by intraclass correlation coefficient (0.5) of pixels. For comparing averages of paired histograms (empirical distribution functions), we applied the KS test again, with adjustment for number of case in each group based on the average of the correlation coefficients between pairs of histograms.

Results

During the study period, 131 subjects were enrolled. However, 3 subjects were not able to return for the PET 2, and 1

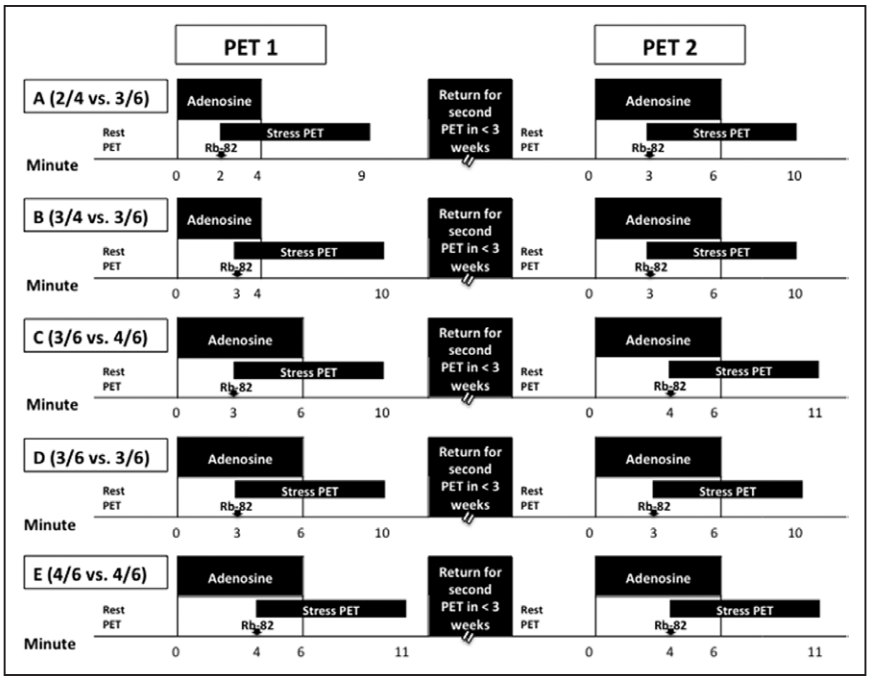

Figure 1. Adenosine infusion protocols. The time-course of various adenosine infusion protocols. Protocol A–C identified the optimum adenosine infusion protocol that produced maximum stress myocardial blood flow. The number prior to slash symbol (/) refers to Rb-82 activation time after adenosine infusion started and the number after slash symbol refers to total adenosine infusion time. For instance, 2/4 refers to 4-minute adenosine infusion protocol with Rb-82 activation at 2 minutes after infusion. Order of paired PETs in each protocol was randomized. For instance, subjects in protocol A could have either 2/4 or 3/6 as PET 1 and 2/4 or 3/6 for PET 2 that did not duplicate PET 1. Test–retest precision was evaluated in protocol D–E, where same subjects underwent 2 PET scans with the same infusion protocol but on a different day. PET indicates positron emission tomography; and Rb-82, rubidium-82.

examination was excluded because of technical error, resulting in 127 subjects with paired data available for further analysis (total of 254 rest–stress PET scans). Baseline characteristics of the subjects are given in Table 1, males comprised 65.4% with average age 54.4±8.8 years; 62.2% had treated dyslipidemia and 41.7% had treated hypertension. Ten subjects (7.9%) had prior revascularization. Median time difference between PET 1 and PET 2 was 11 days (interquartile range 7–21 days). For paired adenosine protocols, 45, 18, and 20 subjects were enrolled to protocols A, B, and C, respectively (Figure 1). To evaluate test–retest precision, 19 subjects underwent similar adenosine infusion protocols (Figure 1, protocols D–E). Finally, 25 subjects were assigned to paired adenosine–dipyridamole protocol (Figure 2).

Adenosine Stress Protocol for Myocardial Blood Flow Quantification

Various adenosine infusion and Rb-82 generator activation times were explored to identify optimal timing that provided maximal hyperemia, shown in Table 2 with paired *t* tests. In the same subject, rest flow was not significantly different between PET 1 and PET 2 in all adenosine infusion protocols. The shorter adenosine infusion protocol at 4 minutes resulted in lower stress flow and CFR when compared with 6-minute infusion protocol for Rb-82 generator activation time at 2 minutes and at 3 minutes of the 4-minute infusion. The 6-minute infusion protocol achieved maximal stress perfusion, with the Rb-82 activation time at 3 minutes or 4 minutes with no significant differences in stress flow or CFR between these 2 timings.

Test–Retest Precision of Myocardial Blood Flow Quantification

Test–retest precision of myocardial blood flow quantification was determined using the same 6-minute adenosine infusion protocol with Rb-82 generator activation at 3 or 4 minutes (protocol D and E) for both PET scans in the same subjects (total of 19 subjects). Rest flow, stress flow, and CFR were not significantly different between PET 1 and PET 2; Table 2. Coefficient of variance for stress flow was ±17% and for CFR was ±20%.

Adenosine Versus Dipyridamole for Myocardial Blood Flow Quantification

The same subject underwent paired PET scans using adenosine and dipyridamole as stress agents (25 subjects in addition to the 102 subjects in Table 2 of subjects with repeated adenosine stress). Figure 3 illustrates no difference in myocardial

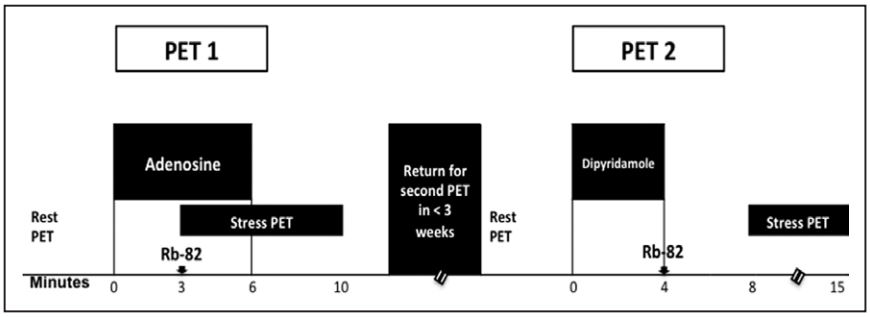

Figure 2. Adenosine vs dipyridamole infusion protocols. To compare stress perfusion and CFR with adenosine and dipyridamole stress, the same subject underwent 2 PET scans using these stressors in randomized order for PET 1 and the other stressor for PET 2 on different day with time course shown. CFR indicates coronary flow reserve; PET, positron emission tomography; and Rb-82, rubidium-82.

Table 1. Baseline Characteristics

	All Subjects (n=127)
Clinical characteristics	
Age, y	54.4±8.8
Sex (male)	83 (65.4)
Body mass index, kg/m^2	29.4±4.9
Coronary artery disease	
Prior bypass surgery	2 (1.6)
Prior percutaneous intervention	8 (6.3)
Prior myocardial infarction	2 (1.6)
Dyslipidemia	79 (62.2)
Diabetes mellitus	8 (6.3)
Hypertension	53 (41.7)
Current smoking	7 (5.5)
Current medications	
Statins	44 (33.6)
ACEI or ARB	37 (29.1)
Antiplatelet use	34 (26.8)
Beta blocker	18 (14.2)
Calcium channel blockers	10 (7.9)
Diuretics	20 (15.8)

Data demonstrated in n (%) or mean±standard deviation. ACEI indicates angiotensin-converting enzyme inhibitor; and ARB, angiotensin receptor blocker.

perfusion and CFR of 25 subjects between rest and paired adenosine versus dipyridamole stress.

Figure 4 illustrates a 57-year-old asymptomatic man with dense coronary calcium in whom submaximal hyperemia using the 4-minute adenosine infusion protocol would result in misinterpretation compared with the 6-minute adenosine protocol. For simplicity, only the lateral and inferior walls are demonstrated. Relative stress images from 4-minute adenosine infusion showed small, mild-to-moderate perfusion defect in the distal inferior wall, while this defect was not present on images with 6-minute adenosine infusion because of higher maximum perfusion (Figure 4A).

Stress myocardial blood flow and CFR were moderate to severely reduced (blue) with the 4-minute adenosine infusion, suggesting diffuse CAD or small vessel disease. However, the 6-minute adenosine images show adequate maximum perfusion (yellow, orange, or red on the stress flow and CFR color bars) well above the low-flow ischemic threshold. There was a mild reduction in stress perfusion in the inferolateral distribution of the distal left circumflex that is also well above the low-flow threshold of ischemia and consistent with mild diffuse CAD associated with coronary calcium. There is perfusion heterogeneity associated with endothelial dysfunction of subclinical CAD (Figure 4B and 4C).[16]

Effect on Regional Ranges of Stress Flow and CFR

Data from myocardial blood flow and CFR are somewhat complex to interpret independently because of perfusion heterogeneity. To integrate stress perfusion in cc/min/g and CFR, both are combined into a single CFC map that is color coded for the same ranges for the above patient groups as previously reported (Figure 4D).[3,4,17] With 4-minute adenosine infusion, CFC is mildly reduced diffusely (yellow on the CFC map color bar) with a small area of moderately reduced CFC in the infero-apex (green). In contrast, the CFC is good (red, orange) for the 6-minute adenosine infusion protocol. Therefore, the 4-minute adenosine infusion that produced only submaximal hyperemia could lead to misinterpreting this study as showing diffuse coronary narrowing, small vessel disease, or a small area of ischemia in the inferior apex. However, with optimal vasodilator stress using the 6-minute adenosine infusion, this study shows good CFC (red, orange).

As reported here, maximum stress perfusion and CFR are essential for demonstrating differences between the 4- and 6-minute adenosine protocols. However, the effects of the 4- versus 6-minute protocols on the entire range of stress perfusion and CFR throughout all pixels of the LV are equally important. Systematically, this comparison over the entire pixel range of perfusion (not just maximum or worst perfusion) requires statistical analysis of the mean histograms for percent of LV in each color-coded severity range of the CFC maps for all the PET studies in each of the 2 stress groups using the KS statistic for comparing histogram distributions.[18]

Table 2. Single Paired Myocardial Rest–Rest, Stress–Stress Perfusion, and CFR–CFR Comparisons From Separate Adenosine Infusion Protocols Each Having Separate Different Patients, by Paired *t* Test

Adenosine Protocols			PET 1 Absolute Flow, cc/min/g, or CFR			PET 2 Absolute Flow, cc/min/g, or CFR			*P* Value		
	PET 1* Rb-82/Adeno	PET 2* Rb-82/Adeno	Rest	Stress	CFR	Rest	Stress	CFR	Rest	Stress	CFR
A (n=45 pairs)	2/4	3/6	0.92±0.26	2.01±0.44	2.30±0.51	0.90±0.26	2.24±0.51	2.66±0.71	0.557	0.001	<0.001
B (n=18 pairs)	3/4	3/6	0.93±0.21	2.03±0.36	2.23±0.52	0.93±0.23	2.32±0.55	2.63±0.67	0.992	0.015	<0.001
C (n=20 pairs)	3/6	4/6	0.88±0.24	2.38±0.44	2.84±0.69	0.86±0.25	2.30±0.44	2.81±0.55	0.437	0.343	0.831
D (n=9 pairs)	3/6	3/6	0.86±0.23	2.01±0.51	2.44±0.58	0.78±0.17	2.01±0.58	2.65±0.72	0.133	0.944	0.259
E (n=10 pairs)	4/6	4/6	1.09±0.22	2.16±0.53	2.04±0.49	1.04±0.30	2.31±0.49	2.33±0.51	0.596	0.296	0.107

CFR indicates coronary flow reserve; PET, positron emission tomography; and Rb-82, rubidium-82.
*Rb-82 generator activation time in minutes/total adenosine infusion time in minutes.

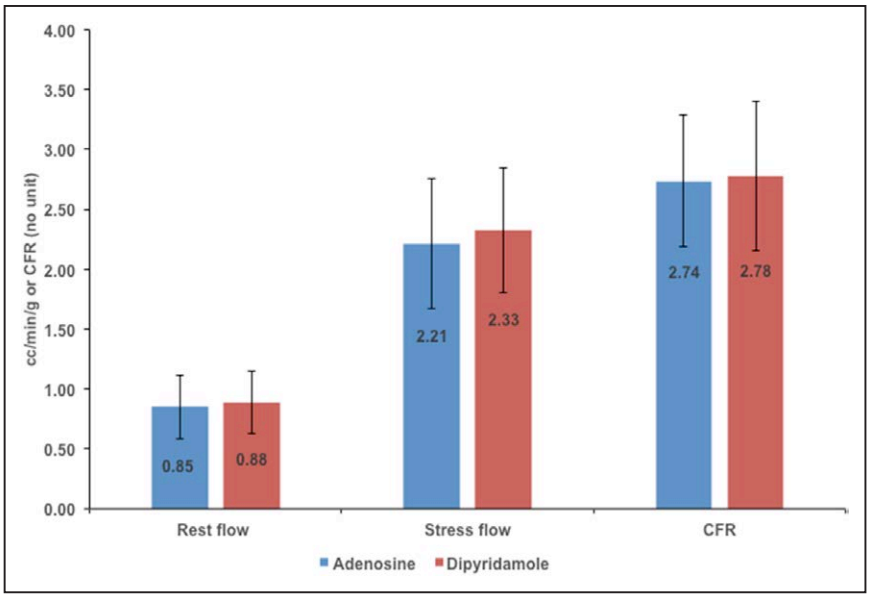

Figure 3. Maximum myocardial perfusion and coronary flow reserve (CFR) using adenosine vs dipyridamole in the same subject. Rest and stress myocardial blood flow and CFR in the same subject undergoing 2 PET scans with adenosine and dipyridamole on different day for 25 paired PETs. Rest flow (P=0.43), stress flow (P=0.23), and CFR (P=0.7) for adenosine and dipyridamole stress were not significantly different by paired t test (N=25). PET indicates positron emission tomography.

Figure 5 shows the primary mean data and Figure 6 the mean CFC histograms for 4- versus 6-minute adenosine protocols. The 6-minute adenosine protocol shows a substantially greater percent of the LV in the highest CFC range and lower percent in the lower ranges compared with the 4-minute protocol.

Figure 7 shows the KS statistic derived from the cumulative probability distribution of each histogram, indicating highly significant differences for 4- versus 6-minute adenosine protocols with $P < e^{-10}$. In contrast to the 11% lower global stress perfusion during submaximal compared with maximum stress, Figure 6 shows that 20% of the 1344 pixels with submaximal stress redistributed into lower flow ranges compared with maximal stress. Therefore, compared with the 6-minute protocol, the 4-minute protocol has more diffusely reduced perfusion capacity, thereby, falsely suggesting more severe CAD or diffuse CAD or microvascular disease that may be misleading because of inadequate stress.

As an additional control comparison, the KS statistic for the 6-minute adenosine versus dipyridamole stress shows no systematic difference (Figure 8A) corresponding to similar global stress perfusion in Figure 3. Separate from the subjects of this study, the KS value for 100 paired, serial, test–retest dipyridamole PETs on different days in the young healthy volunteers is small at 0.01 with no significant difference, thereby, further validating the methodology defining the histogram differences observed between the 2/4 and 3/6 protocols (Figure 8B).

Clinical Importance of Maximal Adenosine Stress

Because data for this adenosine study involved mostly asymptomatic volunteers without CAD, we analyzed whether the lower CFR of 15.7% during the 4-minute adenosine protocol would be clinically important for patients with angina or CAD in real world routine clinical application. Of the 1732 PETs in patients with CAD of our database, 332 (19%) had ≥25% of the LV with CFR ≤2.3 and ≥2.0 where CFR of ≥2.0 is associated with low risk compared with CFR <2.0 associated with high risk.[3,19]

If these 1732 PETs in patients with CAD had quantitative PETs by the 2/4 adenosine protocol, CFR would be on average 15.7% lower to CFR <2.0, thereby, changing these 332 or 19% of these PETs to having CFR <2.0 associated with high risk for coronary events[3,19] but because of suboptimal stress reducing CFR by 15.7% with the 2/4 adenosine protocol. Therefore, potentially, 332 or 19% of these PETs in CAD would show low-flow, high-risk CAD with CFR <2.0 by the 2/4-minute protocol with inappropriate risk stratification, potentially triggering revascularization in some patients.

Discussion

Our study demonstrates that the standard 6-minute adenosine infusion with Rb-82 activation at 3 minutes after infusion caused 15.7% higher CFR and 11.4% higher stress perfusion in cc/min/g compared with 4-minute adenosine infusion with Rb-82 activation at 2 minutes. The entire range of perfusion values and their percent of LV is substantially shifted to low levels quantified by the significant KS statistic, thereby, misleadingly suggesting more severe disease due solely to inadequate stress. Extending adenosine infusion time prior to Rb-82 activation to 3 minutes in the 4-minute adenosine infusion protocol did not result in significant additional increased stress flow or CFR when compared with standard 6-minute adenosine infusion protocol with Rb-82 activation at 3 minutes. In the 6-minute protocol, delaying Rb-82 injection from 3 to 4 minutes provided the same stress perfusion and CFR as the 3-minute injection of Rb-82.

Our results document that the standard stress PET imaging protocol using 6-minute adenosine infusion with Rb-82 activation at 3 minute is the most appropriate adenosine protocol for quantifying maximal myocardial perfusion and CFR comparable to dipyridamole. Blood levels of caffeine were checked in all subjects, with only 2 having low levels of blood caffeine so that no PETs were excluded because of caffeine.

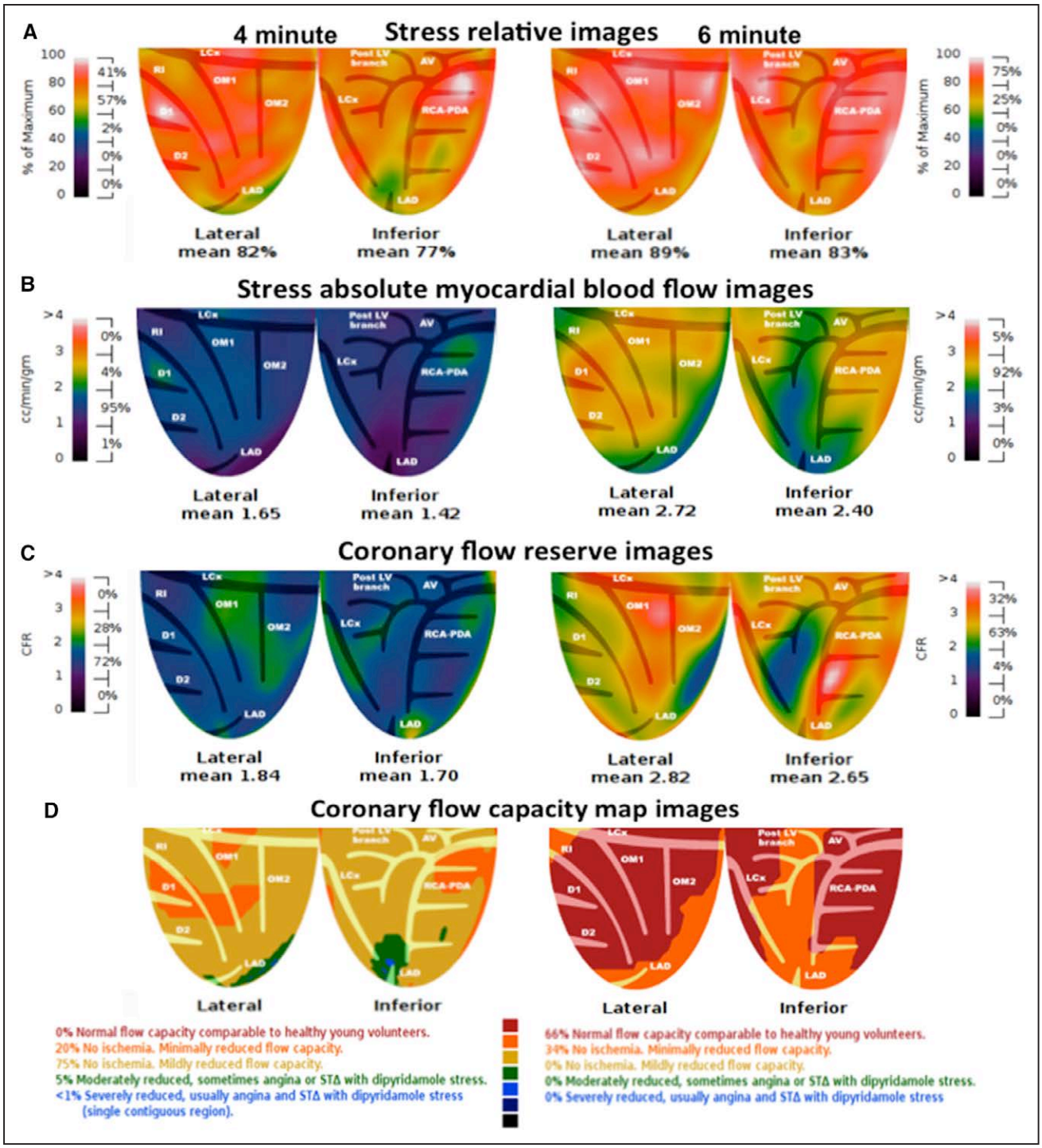

Figure 4. Example of different quantitative result and interpretation for a 4-minute compared with a 6-minute adenosine infusion in the same subject. Submaximal hyperemia using the 4-minute adenosine infusion protocol would result in misinterpretation compared with the 6-minute adenosine protocol. For simplicity, only lateral and inferior walls were demonstrated. Images are scaled by color bar from 100% for maximum relative uptake or flow, with red being next highest and progressively graded to yellow, green, and blue-purple for severe relative defect or severely reduced flow. **A**–**D**, Stress relative images, stress myocardial blood flow images, coronary flow reserve (CFR) images, and coronary flow capacity map images, respectively. Color-coded text gives percent of left ventricle categorized by severity based on the coronary flow capacity map integrating both stress flow and CFR. See article for further discussion. AV indicates atrioventricular node artery; D1, first diagonal; D2, second diagonal; LAD, left anterior descending; LCx, left circumflex; LV, left ventricular; OM1, first obtuse marginal; OM2, second obtuse marginal; PDA, posterior descending artery; RCA, right coronary artery; and RI, Ramus Intermedius.

Paradoxical Effects of Submaximum Stress on Relative Versus Quantitative Perfusion Images

Maximum stress may have apparent opposite effects on relative images compared with absolute stress perfusion and CFR. Relative differences between stenotic and normal coronary arteries are enhanced by maximum stress, thereby, making stress defects on relative images more apparent. However, maximum stress may increase absolute perfusion and CFR in

Color code for severity					
Stress n = 45 pairs severity range	severe	moderate	mild	minimal	good
4 min adenosine average fraction of LV	0.01	0.02	0.31	0.45	0.21
6 min adensoine average fraction of LV	0.00	0.01	0.19	0.39	0.42
4 min adenosine cumulative probability	0.01	0.03	0.34	0.79	1.00
6 min adenosine cumulative probability	0.00	0.01	0.20	0.59	1.00

Figure 5. Average fraction of left ventricle (LV) in each range of color-coded severity for coronary flow capacity maps of 4-minute vs 6-minute adenosine stress.

both the stenotic and normal artery compared with submaximal stress (except for myocardial steal).

Submaximal stress causes the opposite, that is, less severe stress defect on relative images but lower absolute stress perfusion and CFR that implies more severe CAD because of submaximum stress compared with normal stress perfusion and CFR with maximal stress. Thus, compared with maximal stress with the 3/6 protocol, submaximal stress yielding low stress perfusion and CFR may make mildly reduced quantitative perfusion by the 3/6 protocol look severe by the 2/4 protocol, mimicking diffuse or microvascular disease, hence, potentially false-positive severity as in Figure 4.

Test–retest precision of myocardial blood flow quantification in our study (coefficient of variance of ≈20%) is comparable to prior cardiac PET studies using Rb-82, N-13, or O-15 as radiotracers.[3,10,14,17,19–21] Total radiation dose for two 3-PET sequences was 16 mSv, comparable to an average Tc99m Sestimibi rest stress study.[22]

To our knowledge, this article reports a first application in cardiovascular medicine of the KS analysis for differences in histogram distributions of 1344 pixel measurements of CFC of the LV, quantifying focal and diffuse CAD.

Potential Mechanisms for Suboptimal Adenosine Stress

Continuous measurements of coronary pressure after intravenous adenosine provide a mechanism for the 4-minute adenosine causing submaximal quantitative perfusion[23] because of transient minimum coronary pressure for FFR. In 24% to 29% of intracoronary pressure tracings, coronary pressure transiently falls for the FFR measurement then returns to baseline and continues to fluctuate within minutes[23] after intravenous adenosine. These fluctuating pressures reflect fluctuating submaximal flow observed separately by intracoronary Doppler in other studies that is reduced by higher doses,[24] paralleling our observations on submaximum adenosine stress with the 2/4-minute protocol, possibly related to incomplete A2 receptor saturation.

Limitations

The average 15.7% significant difference in CFR between the 2/4 and 3/6 adenosine protocols incorporates the reported average±21% variability of quantitative PET,[3,21] indicating that significant average differences observed between groups may not be seen in individual cases. However, for some patients, the 3/6 protocol may provide essential information for personalized management. Further investigation in future studies is required for determining the impact of differences in maximum blood flow with different stress protocols on risk stratification and outcomes after revascularization procedures based on quantitative perfusion. Although a larger sample size may yield different results, the sample size reported is adequate for highly significant results, with P values <0.001.

Conclusions

Among 127 volunteer subjects with paired quantitative myocardial perfusion by Rb-82 PET and various adenosine infusion protocols, the 6-minute adenosine infusion provided maximum myocardial perfusion comparable to a 4-minute dipyridamole infusion with Rb-82 injection at 8 minutes. The 6-minute adenosine infusion with Rb-82 activation at

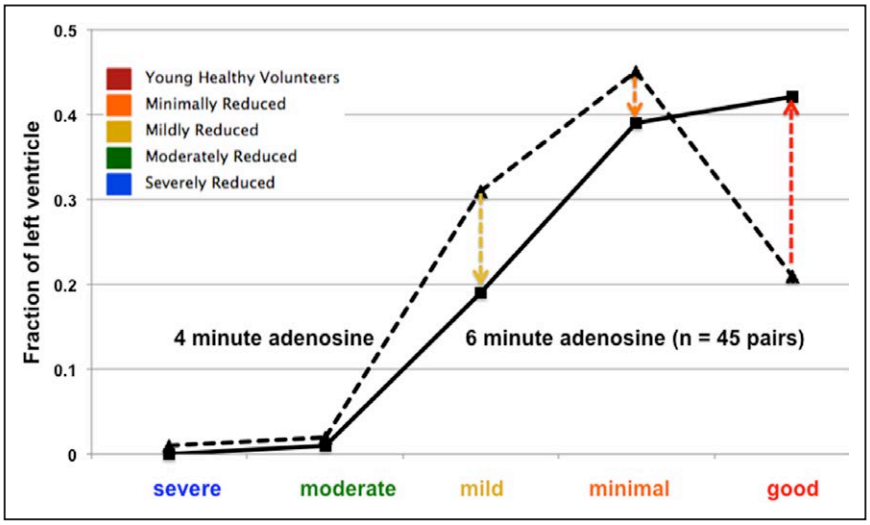

Figure 6. Histograms of the average fraction of LV in color-coded severity ranges of coronary flow capacity for adenosine 4-minute vs 6-minute stress protocol. Dashed arrows indicate comparative change between adenosine and dipyridamole in each color coded range of severity.

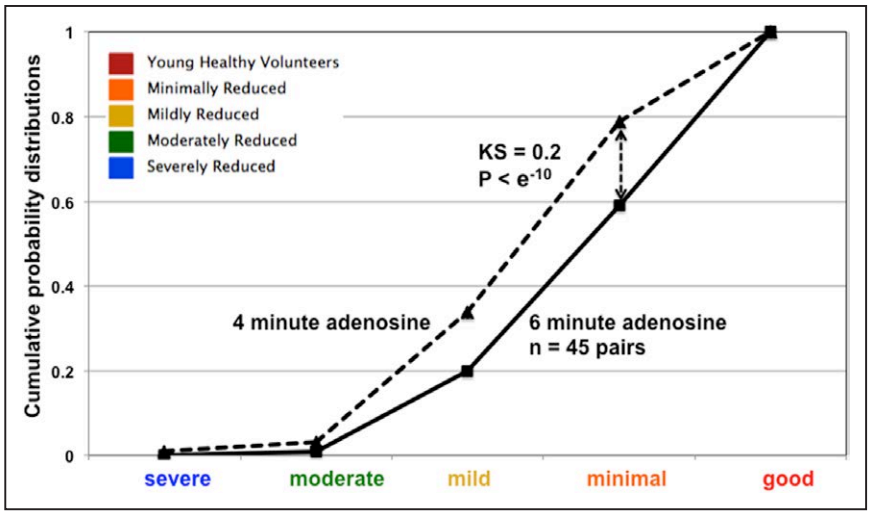

Figure 7. Mean difference in severity distribution histograms of coronary flow capacity for adenosine 4- vs 6-minute protocol—the Kolmogorov–Smirnov statistic. Dashed arrow indicated maximal difference between 2 cumulative histograms that determines statistical significance of their difference.

3 minutes increased CFR and maximum stress perfusion by 15.7% and 11.4%, respectively, more than the 4-minute adenosine protocol with Rb-82 injection at 2 minutes. The optimal stress substantially reduced perfusion heterogeneity and increased perfusion throughout the entire histogram range of perfusion as percent of the LV that was highly

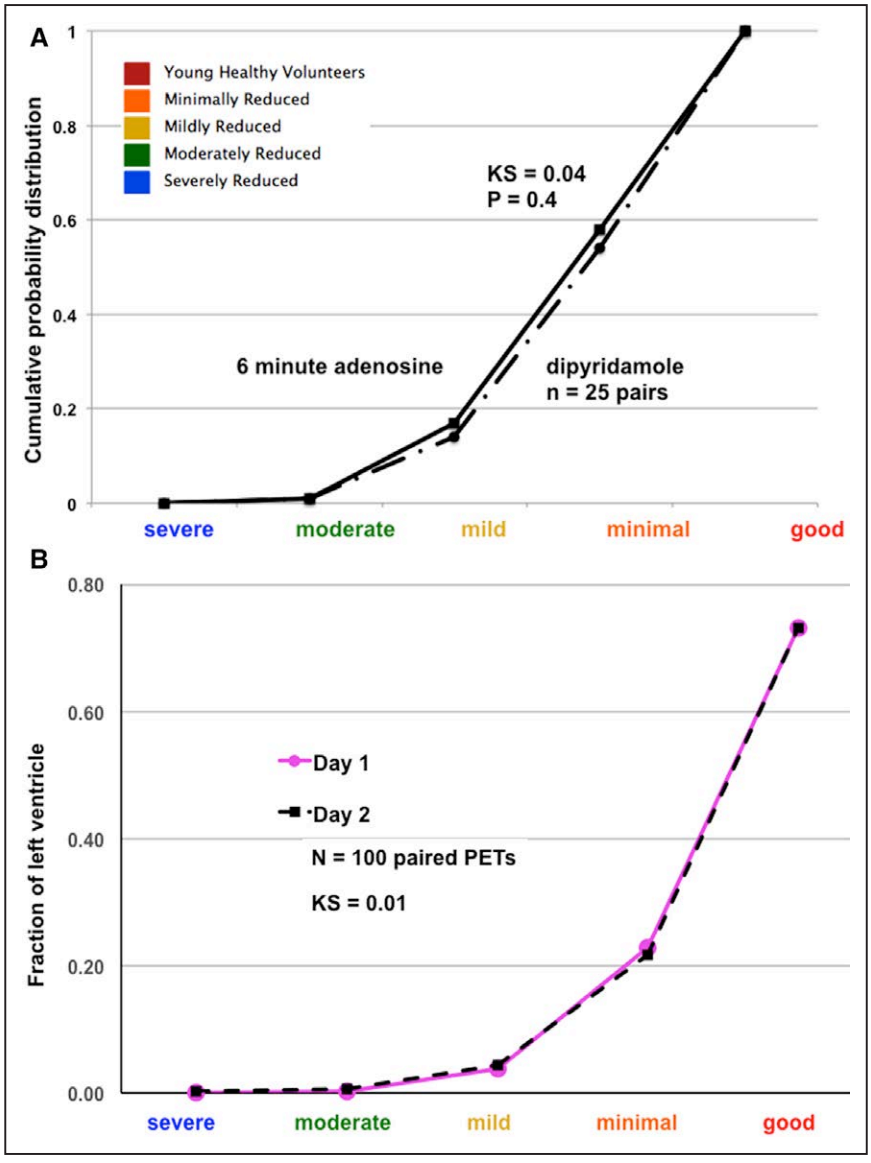

Figure 8. Control paired PET histogram comparisons. **A**, The mean difference in severity distribution histograms of coronary flow capacity for adenosine 6 minute vs dipyridamole stress by Kolmogorov–Smirnov (KS) statistic shows no difference (*P*=0.4). **B**, Mean difference in severity distribution histograms of coronary flow capacity for 100 serial paired dipyridamole stress PETs in healthy young volunteers by Kolmogorov–Smirnov statistic shows no difference (*P*=0.9). PET indicates positron emission tomography.

significant by KS analysis uniquely applied in cardiovascular medicine.

The 4-minute adenosine infusion with Rb-82 activation at 2 minutes produced CFR that averaged 15.7% lower than the 3/6 minute protocol. Consequently, in a CAD cohort separate from volunteers of this study, compared with the 3/6-minute protocol, the 2/4-minute adenosine protocol would potentially have changed 332 of 1732 (19%) PETs at low-risk physiological severity CFR ≥2.3 to CFR <2.0, implying high-risk quantitative severity, potentially leading to interventions but as a result of suboptimal stress of the 2/4 protocol in some patients. Therefore, for these patients, the 3/6 protocol may provide essential information for personalized management.

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References

1. Hendel RC, Berman DS, Di Carli MF, Heidenreich PA, Henkin RE, Pellikka PA, Pohost GM, Williams KA; American College of Cardiology Foundation Appropriate Use Criteria Task Force; American Society of Nuclear Cardiology; American College of Radiology; American Heart Association; American Society of Echocardiography; Society of Cardiovascular Computed Tomography; Society for Cardiovascular Magnetic Resonance; Society of Nuclear Medicine. ACCF/ASNC/ACR/AHA/ASE/SCCT/SCMR/SNM 2009 appropriate use criteria for cardiac radionuclide imaging: a report of the American College of Cardiology Foundation Appropriate Use Criteria Task Force, the American Society of Nuclear Cardiology, the American College of Radiology, the American Heart Association, the American Society of Echocardiography, the Society of Cardiovascular Computed Tomography, the Society for Cardiovascular Magnetic Resonance, and the Society of Nuclear Medicine. *Circulation*. 2009;119:e561–e587. doi: 10.1161/CIRCULATIONAHA.109.192519.
2. Henzlova MJ, Duvall WL, Einstein AJ, Travin MI, Verberne HJ. ASNC imaging guidelines for SPECT nuclear cardiology procedures: Stress, protocols, and tracers. *J Nucl Cardiol*. 2016;23:606–639. doi: 10.1007/s12350-015-0387-x.
3. Gould KL, Johnson NP, Bateman TM, Beanlands RS, Bengel FM, Bober R, Camici PG, Cerqueira MD, Chow BJ, Di Carli MF, Dorbala S, Gewirtz H, Gropler RJ, Kaufmann PA, Knaapen P, Knuuti J, Merhige ME, Rentrop KP, Ruddy TD, Schelbert HR, Schindler TH, Schwaiger M, Sdringola S, Vitarello J, Williams KA Sr, Gordon D, Dilsizian V, Narula J. Anatomic versus physiologic assessment of coronary artery disease. Role of coronary flow reserve, fractional flow reserve, and positron emission tomography imaging in revascularization decision-making. *J Am Coll Cardiol*. 2013;62:1639–1653. doi: 10.1016/j.jacc.2013.07.076.
4. Gould KL, Johnson NP, Kaul S, Kirkeeide RL, Mintz GS, Rentrop KP, Sdringola S, Virmani R, Narula J. Patient selection for elective revascularization to reduce myocardial infarction and mortality: new lessons from randomized trials, coronary physiology, and statistics. *Circ Cardiovasc Imaging*. 2015;8e003099. doi: 10.1161/CIRCIMAGING.114.003099.
5. Boden WE, O'Rourke RA, Teo KK, Hartigan PM, Maron DJ, Kostuk WJ, Knudtson M, Dada M, Casperson P, Harris CL, Chaitman BR, Shaw L, Gosselin G, Nawaz S, Title LM, Gau G, Blaustein AS, Booth DC, Bates ER, Spertus JA, Berman DS, Mancini GB, Weintraub WS; COURAGE Trial Research Group. Optimal medical therapy with or without PCI for stable coronary disease. *N Engl J Med*. 2007;356:1503–1516. doi: 10.1056/NEJMoa070829.
6. De Bruyne B, Fearon WF, Pijls NH, Barbato E, Tonino P, Piroth Z, Jagic N, Mobius-Winckler S, Rioufol G, Witt N, Kala P, MacCarthy P, Engström T, Oldroyd K, Mavromatis K, Manoharan G, Verlee P, Frobert O, Curzen N, Johnson JB, Limacher A, Nüesch E, Jüni P; FAME 2 Trial Investigators. Fractional flow reserve-guided PCI for stable coronary artery disease. *N Engl J Med*. 2014;371:1208–1217. doi: 10.1056/NEJMoa1408758.
7. van Nunen LX, Zimmermann FM, Tonino PA, Barbato E, Baumbach A, Engstrøm T, Klauss V, MacCarthy PA, Manoharan G, Oldroyd KG, Ver Lee PN, Van't Veer M, Fearon WF, De Bruyne B, Pijls NH; FAME Study Investigators. Fractional flow reserve versus angiography for guidance of PCI in patients with multivessel coronary artery disease (FAME): 5-year follow-up of a randomised controlled trial. *Lancet*. 2015;386:1853–1860. doi: 10.1016/S0140-6736(15)00057-4.
8. Tonino PA, De Bruyne B, Pijls NH, Siebert U, Ikeno F, van' t Veer M, Klauss V, Manoharan G, Engstrøm T, Oldroyd KG, Ver Lee PN, MacCarthy PA, Fearon WF; FAME Study Investigators. Fractional flow reserve versus angiography for guiding percutaneous coronary intervention. *N Engl J Med*. 2009;360:213–224. doi: 10.1056/NEJMoa0807611.
9. De Bruyne B, Baudhuin T, Melin JA, Pijls NH, Sys SU, Bol A, Paulus WJ, Heyndrickx GR, Wijns W. Coronary flow reserve calculated from pressure measurements in humans. Validation with positron emission tomography. *Circulation*. 1994;89:1013–1022.
10. Johnson NP, Gould KL. Regadenoson versus dipyridamole hyperemia for cardiac PET imaging. *JACC Cardiovasc Imaging*. 2015;8:438–447. doi: 10.1016/j.jcmg.2014.11.016.
11. Sdringola S, Johnson NP, Kirkeeide RL, Cid E, Gould KL. Impact of unexpected factors on quantitative myocardial perfusion and coronary flow reserve in young, asymptomatic volunteers. *JACC Cardiovasc Imaging*. 2011;4:402–412. doi: 10.1016/j.jcmg.2011.02.008.
12. Gould KL, Pan T, Loghin C, Johnson NP, Sdringola S. Reducing radiation dose in rest-stress cardiac PET/CT by single poststress cine CT for attenuation correction: quantitative validation. *J Nucl Med*. 2008;49:738–745. doi: 10.2967/jnumed.107.049163.
13. Yoshida K, Mullani N, Gould KL. Coronary flow and flow reserve by PET simplified for clinical applications using rubidium-82 or nitrogen-13-ammonia. *J Nucl Med*. 1996;37:1701–1712.
14. Renaud JM, DaSilva JN, Beanlands RS, DeKemp RA. Characterizing the normal range of myocardial blood flow with ^{82}rubidium and ^{13}N-ammonia PET imaging. *J Nucl Cardiol*. 2013;20:578–591. doi: 10.1007/s12350-013-9721-3.
15. Vasquez AF, Johnson NP, Gould KL. Variation in quantitative myocardial perfusion due to arterial input selection. *JACC Cardiovasc Imaging*. 2013;6:559–568. doi: 10.1016/j.jcmg.2012.11.015.
16. Johnson NP, Gould KL. Physiology of endothelin in producing myocardial perfusion heterogeneity: a mechanistic study using darusentan and positron emission tomography. *J Nucl Cardiol*. 2013;20:835–844. doi: 10.1007/s12350-013-9756-5.
17. Johnson NP, Gould KL. Integrating noninvasive absolute flow, coronary flow reserve, and ischemic thresholds into a comprehensive map of physiological severity. *JACC Cardiovasc Imaging*. 2012;5:430–440. doi: 10.1016/j.jcmg.2011.12.014.
18. Conover WJ. *Practical Nonparametric Statistics*, 3rd edition. New York: Wiley; 1999.
19. Taqueti VR, Hachamovitch R, Murthy VL, Naya M, Foster CR, Hainer J, Dorbala S, Blankstein R, Di Carli MF. Global coronary flow reserve is associated with adverse cardiovascular events independently of luminal angiographic severity and modifies the effect of early revascularization. *Circulation*. 2015;131:19–27. doi: 10.1161/CIRCULATIONAHA.114.011939.
20. Nagamachi S, Czernin J, Kim AS, Sun KT, Böttcher M, Phelps ME, Schelbert HR. Reproducibility of measurements of regional resting and hyperemic myocardial blood flow assessed with PET. *J Nucl Med*. 1996;37:1626–1631.
21. Kitkungvan D, Johnson NP, Roby NP, Patel MB, Kirkeeide R, Gould KL. Routine clinical quantitative rest stress myocardial perfusion for managing coronary artery disease: clinical relevance of test-retest variability [published online ahead of print December 21, 2016]. *JACC Cardiovasc Imaging*. 2016. doi: 10.1016/j.jcmg.2016.09.019.
22. Jerome SD, Tilkemeier PL, Farrell MB, Shaw LJ. Nationwide Laboratory Adherence to Myocardial Perfusion Imaging Radiation Dose Reduction Practices: A Report From the Intersocietal Accreditation Commission

Data Repository. *JACC Cardiovasc Imaging*. 2015;8:1170–1176. doi: 10.1016/j.jcmg.2015.07.008.
23. Johnson NP, Johnson DT, Kirkeeide RL, Berry C, De Bruyne B, Fearon WF, Oldroyd KG, Pijls NH, Gould KL. Repeatability of Fractional Flow Reserve Despite Variations in Systemic and Coronary Hemodynamics. *JACC Cardiovasc Interv*. 2015;8:1018–1027. doi: 10.1016/j.jcin.2015.01.039.
24. Wilson RF, Wyche K, Christensen BV, Zimmer S, Laxson DD. Effects of adenosine on human coronary arterial circulation. *Circulation*. 1990;82:1595–1606.

CLINICAL PERSPECTIVE

Revascularization procedures are increasingly guided by physiological severity of coronary stenosis quantified by relative or absolute coronary flow reserve (CFR) using positron emission tomography, magnetic resonance imaging, or intracoronary pressure wires during vasodilator stress. Just as exercise stress testing to heart rate <75% of maximum reduces its diagnostic accuracy, suboptimal vasodilator stress also reduces diagnostic accuracy. The 4-minute adenosine protocol with radionuclide injection at 2 minutes yields CFR that significantly averaged 15.7% lower than the 6-minute adenosine protocol with radionuclide injection at 3 minutes. In a coronary artery disease cohort separate from volunteers of this study, compared with the 3/6-minute protocol, the 2/4-minute adenosine protocol would potentially have changed 332 of 1732 (19%) positron emission tomographies at low-risk physiological severity CFR ≥2.3 to CFR <2.0, thereby implying high-risk quantitative severity potentially appropriate for interventions but as a result of suboptimal stress of the 2/4 protocol in some patients. The average 15.7% reduction in CFR for the 2/4 adenosine protocol versus the 3/6 protocol incorporates the average ±21% variability of serial quantitative positron emission tomography, indicating that significant average differences observed between groups may not apply to individual cases. However, for some patients, the 3/6 protocol may provide essential information for personalized management.

Optimal Adenosine Stress for Maximum Stress Perfusion, Coronary Flow Reserve, and Pixel Distribution of Coronary Flow Capacity by Kolmogorov–Smirnov Analysis

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