SUPPLEMENTAL MATERIAL

SUPPLEMENTAL METHODS

1) Positron Emission Tomography Protocol

Transmission CT scans were first performed for attenuation correction purposes. Intravenous bolus administration of ^{13}N-ammonia (~20 mCi) was performed simultaneously with list mode imaging acquisition over 20 minutes. Thirty minutes after completion of the rest images, intravenous regadenoson (0.4 mg) was infused over 30 seconds. Thirty seconds after completion of the regadenoson administration, a second dose of ^{13}N-ammonia was injected and images were recorded in the same manner. Heart rate, systemic blood pressure, and 12-lead electrocardiogram were recorded at baseline and every minute after regadenoson infusion.

2) Assessment For Scatter From Liver And Attentuation Correction Using An Anthropomorphic Phanton

We performed a phantom study to assess the biases in global and regional retention and myocardial perfusion reserve index (MPR index) estimates due to (1) scatter from liver activity and (2) lack of attenuation correction. The Data Spectrum Anthropomorphic Torso Phantom with cardiac insert was scanned on the D-SPECT camera under two conditions: Tc-99m activity in the cardiac insert only and Tc-99m activity in both the cardiac insert and the liver. Cardiac and liver activity concentrations were equal. Attenuation correction was performed by using a CT image acquired on a separate CT scanner. The CT image was registered to the SPECT image with an automatic rigid alignment algorithm and then converted into an attenuation coefficients map using a tri-linear transformation dependent on CT & SPECT photons energies. Then, the resulted attenuation coefficient map was filtered with a gaussian filter to match SPECT resolution, and to be used within the SPECT iterative reconstruction projection phase for correcting the pixels attenuation effect, as described previously[25-27].

Reconstructed images were segmented using Corridor 4DM (INVIA Medical Imaging Solutions, Ann Arbor, Michigan). Comparison of cardiac-only and cardiac-and-liver images, after corrections for decay, yielded estimates of the contributions of liver activity to global myocardium and regional segments for equal activity concentrations. We assumed that effects of liver scatter on the LV blood pool were the same as for the global myocardium. The relative activity concentrations of

myocardium and liver over time under rest conditions were obtained from published data for ^{94}Tc-MIBI PET dynamic images[19]; we assumed the same relationships for stress conditions. We also assumed that the contribution of liver activity to cardiac activity over time, determined for equal activity concentrations by the phantom experiment, was proportional to the published liver-to-heart ratio. This enabled us to calculate "scatter-free" time-activity curves for the LV blood pool, global myocardium, and the three segments. Similarly, comparison of attenuation-corrected and non-attenuation-corrected heart-and-liver images yielded estimates of the biases due to lack of attenuation correction and enabled us to calculate "attenuation-corrected" time-activity curves for the same regions. "Scatter-free" and "attenuation-corrected" TAC were calculated for 4 patients, for rest and stress conditions, using standard and spline-fitted reconstructions.

Mean K1 percentage differences between cardiac-only and cardiac-and-liver data in spline-fitted images were 5.85 ± 0.17% for global rest K1, 7.67 ± 0.45% for global stress K1 and 1.93 ± 0.54% for the stress/rest K1 ratio (myocardial perfusion reserve) (**Table S3**). Percentage differences of similar magnitude were observed for regional values during rest (5.74 ± 0.18% in LAD, 6.16 ± 0.15% in LCX, and 6.59 ± 0.17% in RCA territories), and stress (7.61 ± 0.57% in LAD, 8.30 ± 0.38% in LCX and 8.32 ± 0.71% in the RCA territories). The results were essentially the same for standard OSEM reconstruction; mean global K1 percentage differences between cardiac-only and cardiac-and-liver data in spline-fitted images were 5.71 ± 0.24% for global rest K1, 7.18 ± 0.86% for global stress K1 and 1.57 ± 0.70% for the stress/rest K1 ratio (myocardial perfusion reserve). Similar results were obtained for regional rest K1 (5.62 ± 0.25% in LAD, 6.03 ± 0.27% in LCX, and 6.41 ± 0.25% in RCA territories), and stress K1 (7.11 ± 1.04% in LAD, 7.40 ± 0.28% in LCX, and 8.24 ± 1.50 in RCA territories).

We used the same sample of four patients to assess the impact of attenuation correction application on the global and regional retention values. The correction for attenuation led to relatively small changes in K1 and MPR index values independently of the reconstruction algorithm used (**Table S4**). Mean percentage difference between spline-fitted non-attenuation-corrected and attenuated-corrected images were 3.62 ± 0.10% for global rest K1, 4.72 ± 0.29% for global stress K1, and 1.15 ± 0.33% for global MPR index (stress/rest K1 ratio). Similar results were obtained for standard OSEM reconstruction, with 3.53 ± 0.15% difference for global rest K1, 4.43 ± 0.54% for global stress K1 and 0.93 ± 0.42% for global MPR index.

Larger clinical studies are needed to assess the need for scatter, attenuation, and motion correction in dynamic SPECT.

SUPPLEMENTAL FIGURES

FIGURE S1

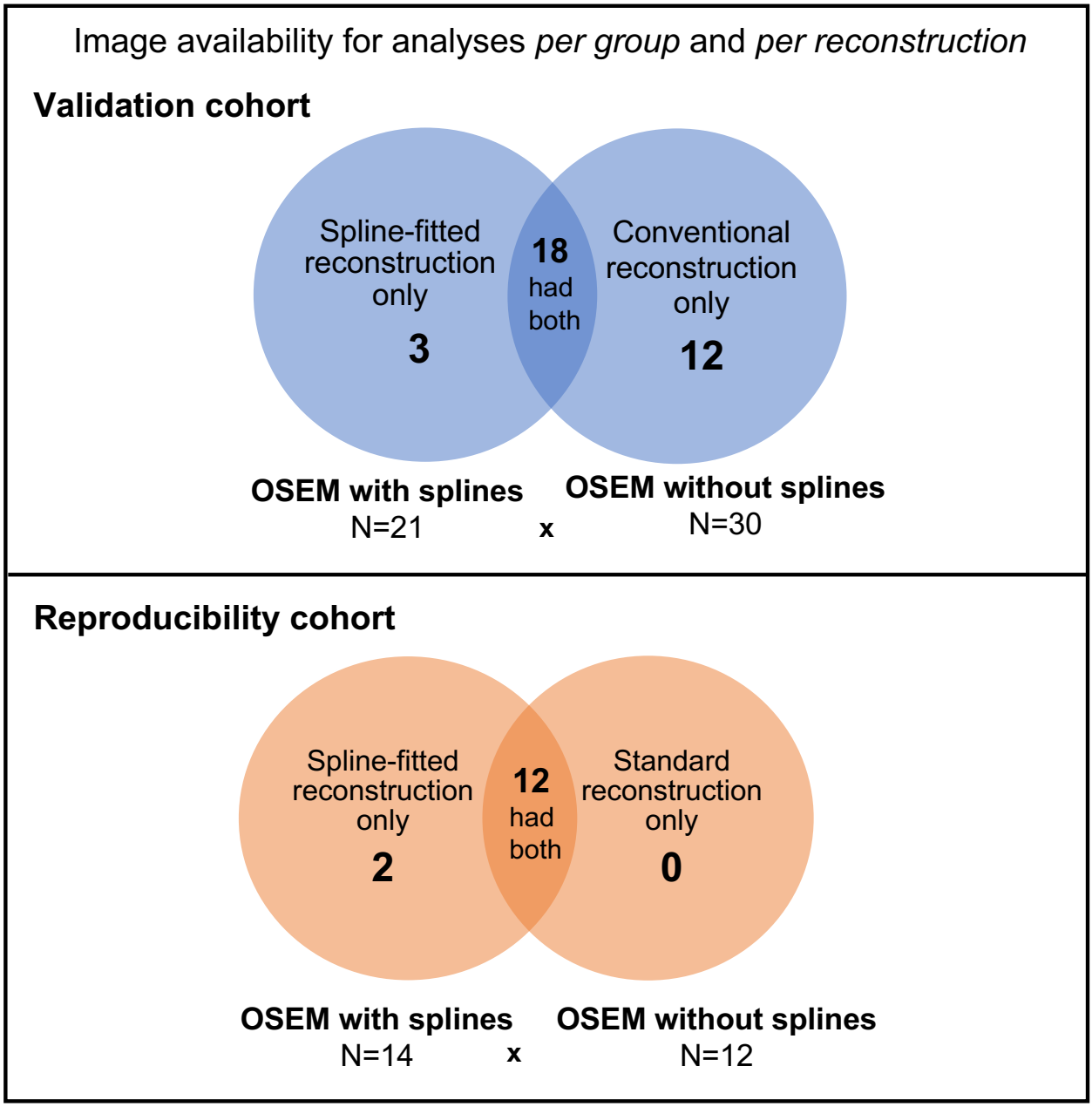


FIGURE S2

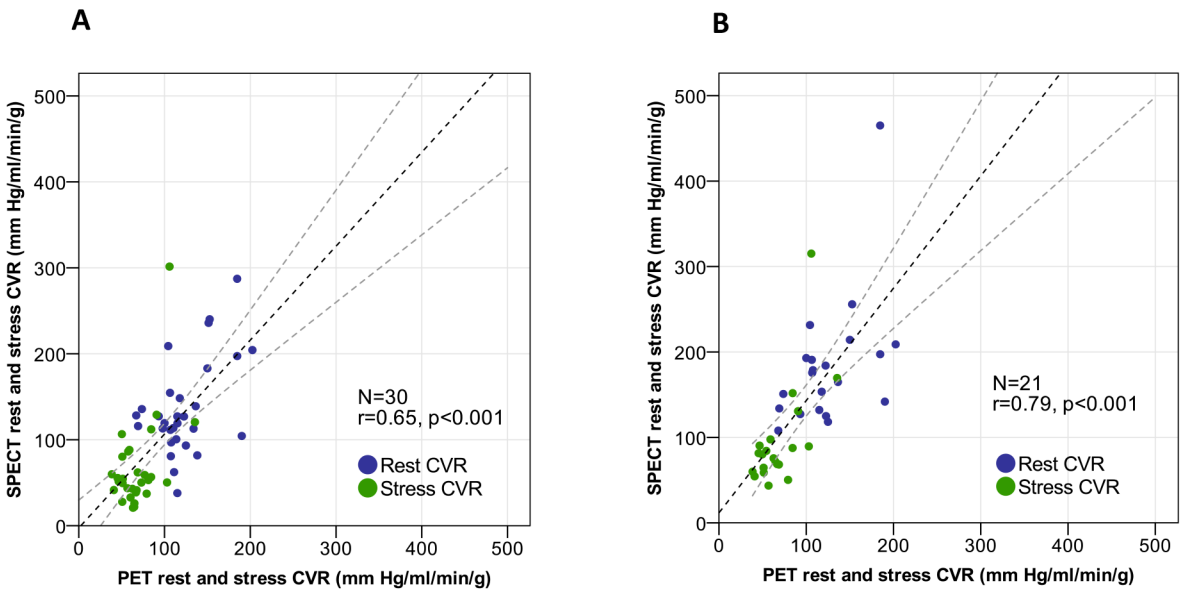

A
SPECT rest and stress CVR (mm Hg/ml/min/g)
PET rest and stress CVR (mm Hg/ml/min/g)
N=30
r=0.65, p<0.001
Rest CVR
Stress CVR
0
100
200
300
400
500
B
SPECT rest and stress CVR (mm Hg/ml/min/g)
PET rest and stress CVR (mm Hg/ml/min/g)
N=21
r=0.79, p<0.001
Rest CVR
Stress CVR

FIGURE S3

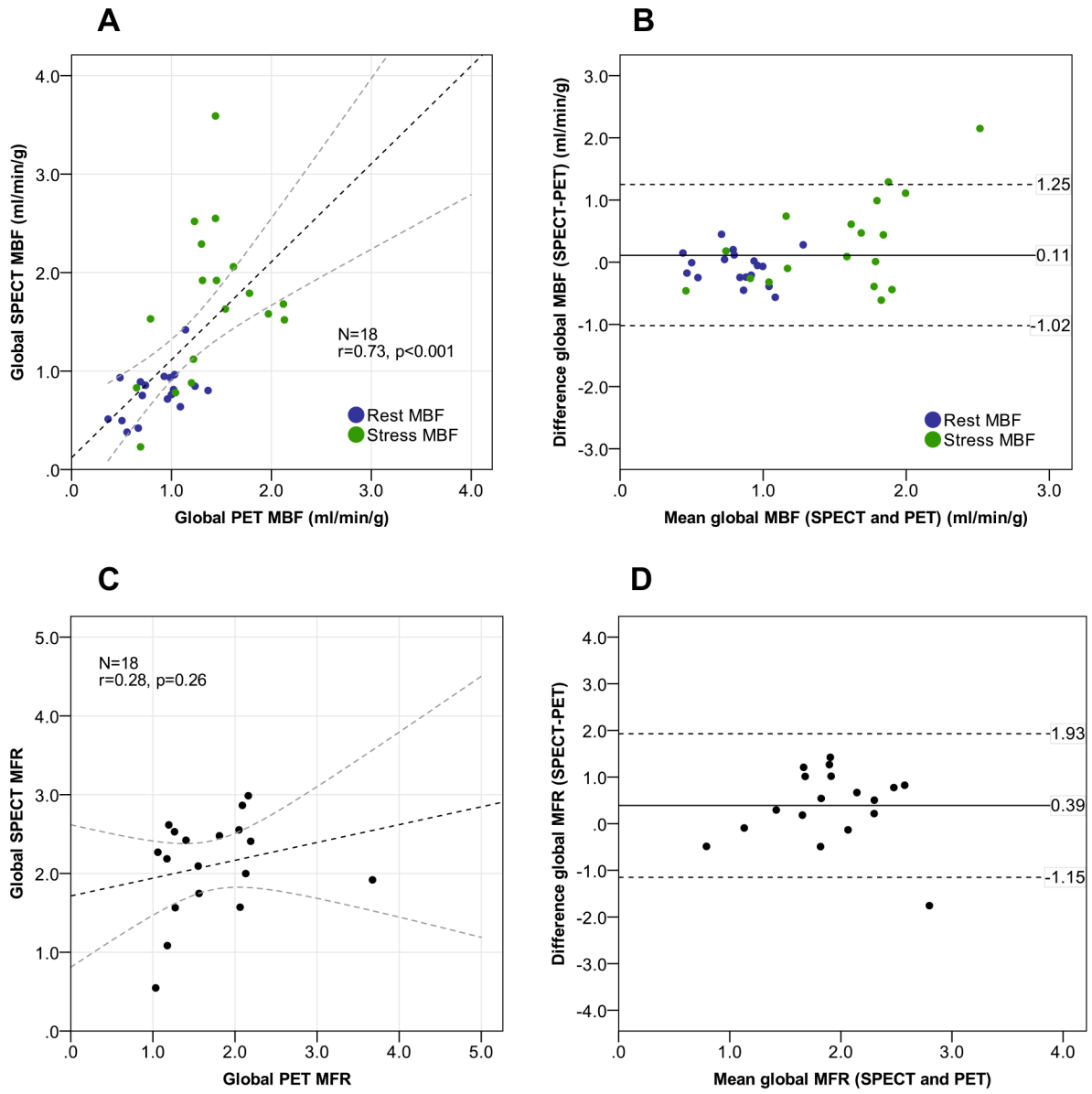

A
Global SPECT MBF (ml/min/g)
Global PET MBF (ml/min/g)
N=18
r=0.73, p<0.001
Rest MBF
Stress MBF
B
Difference global MBF (SPECT-PET) (ml/min/g)
Mean global MBF (SPECT and PET) (ml/min/g)
1.25
0.11
-1.02
Rest MBF
Stress MBF
C
Global SPECT MFR
Global PET MFR
N=18
r=0.28, p=0.26
D
Difference global MFR (SPECT-PET)
Mean global MFR (SPECT and PET)
1.93
0.39
-1.15

FIGURE S4

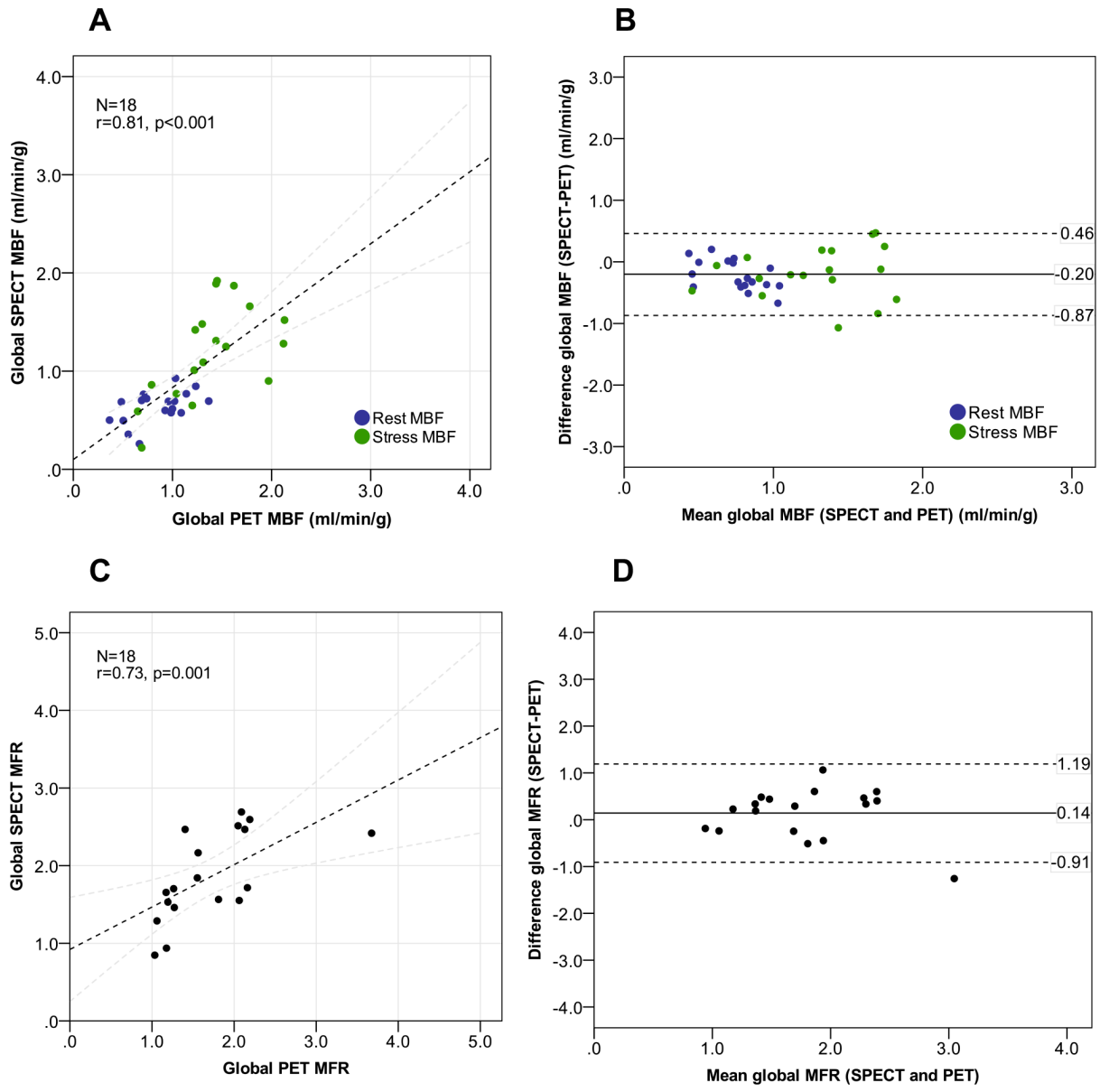

A
N=18
r=0.81, p<0.001
Global SPECT MBF (ml/min/g)
Global PET MBF (ml/min/g)
Rest MBF
Stress MBF
B
Difference global MBF (SPECT-PET) (ml/min/g)
Mean global MBF (SPECT and PET) (ml/min/g)
0.46
-0.20
-0.87
Rest MBF
Stress MBF
C
N=18
r=0.73, p=0.001
Global SPECT MFR
Global PET MFR
D
Difference global MFR (SPECT-PET)
Mean global MFR (SPECT and PET)
1.19
0.14
-0.91

FIGURE S5

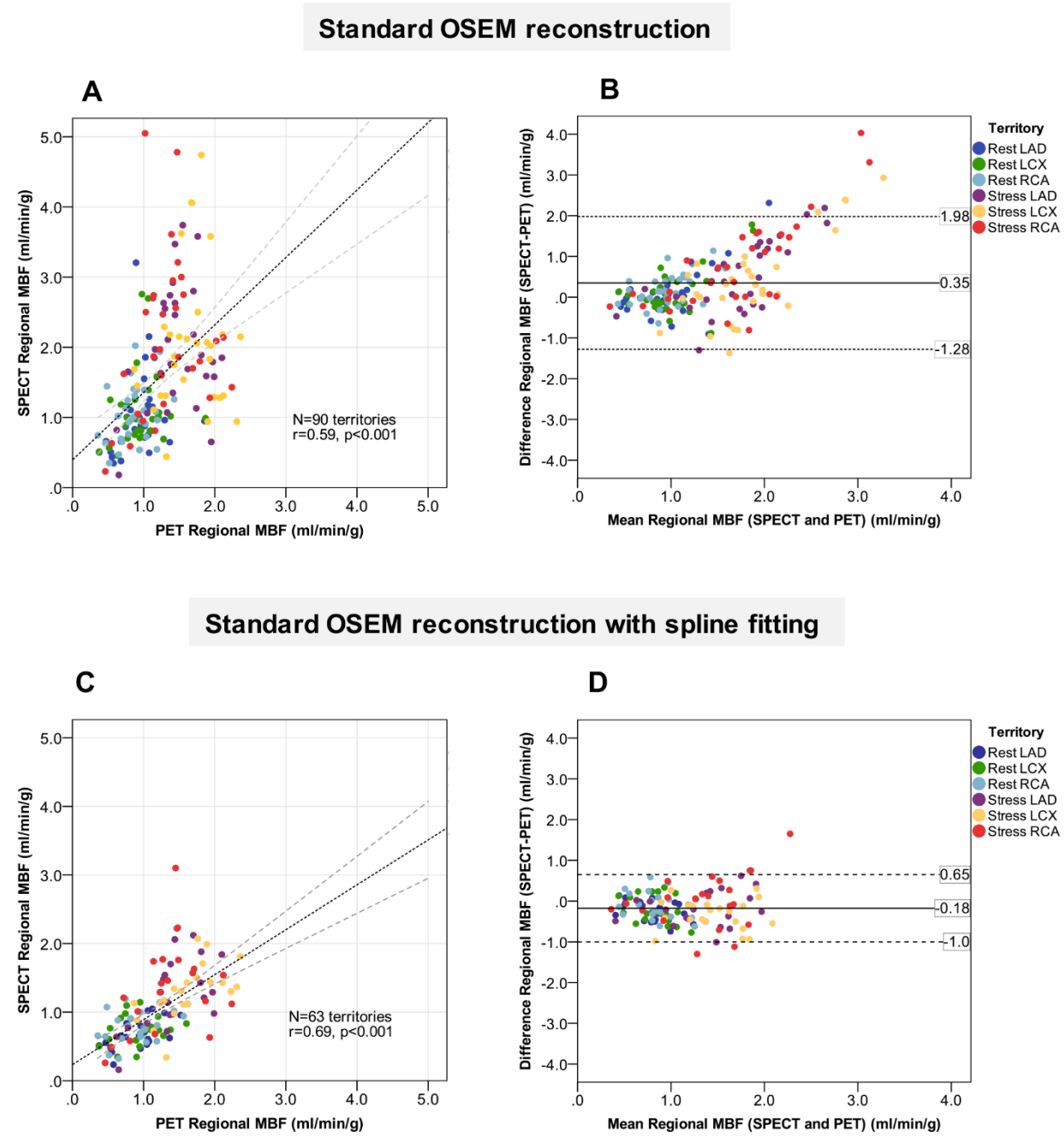

Standard OSEM reconstruction
A
SPECT Regional MBF (ml/min/g)
PET Regional MBF (ml/min/g)
N=90 territories
r=0.59, p<0.001
B
Difference Regional MBF (SPECT-PET) (ml/min/g)
Mean Regional MBF (SPECT and PET) (ml/min/g)
1.98
0.35
-1.28
Territory
Rest LAD
Rest LCX
Rest RCA
Stress LAD
Stress LCX
Stress RCA
Standard OSEM reconstruction with spline fitting
C
SPECT Regional MBF (ml/min/g)
PET Regional MBF (ml/min/g)
N=63 territories
r=0.69, p<0.001
D
Difference Regional MBF (SPECT-PET) (ml/min/g)
Mean Regional MBF (SPECT and PET) (ml/min/g)
0.65
-0.18
-1.0
Territory
Rest LAD
Rest LCX
Rest RCA
Stress LAD
Stress LCX
Stress RCA

FIGURE S6

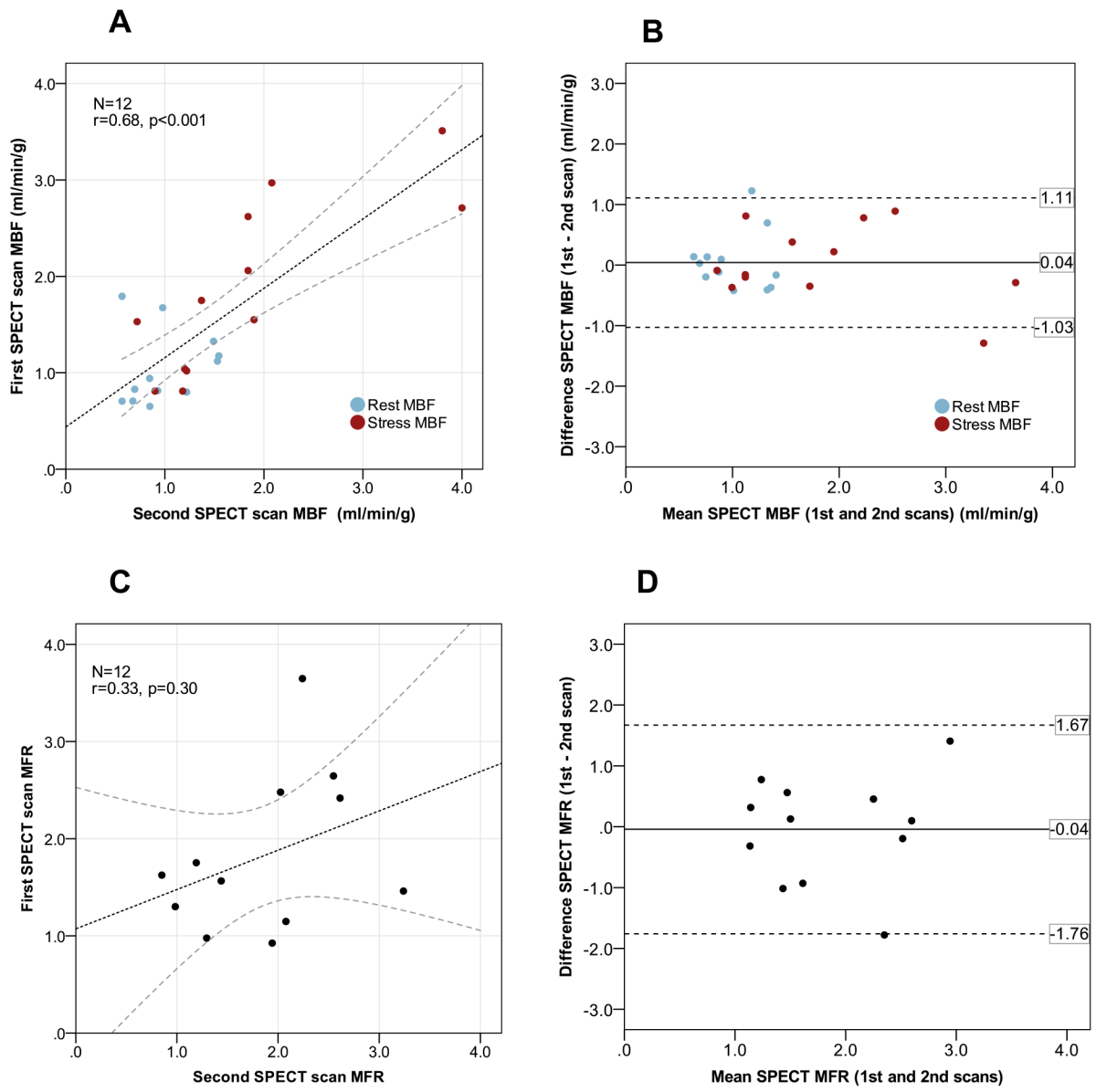
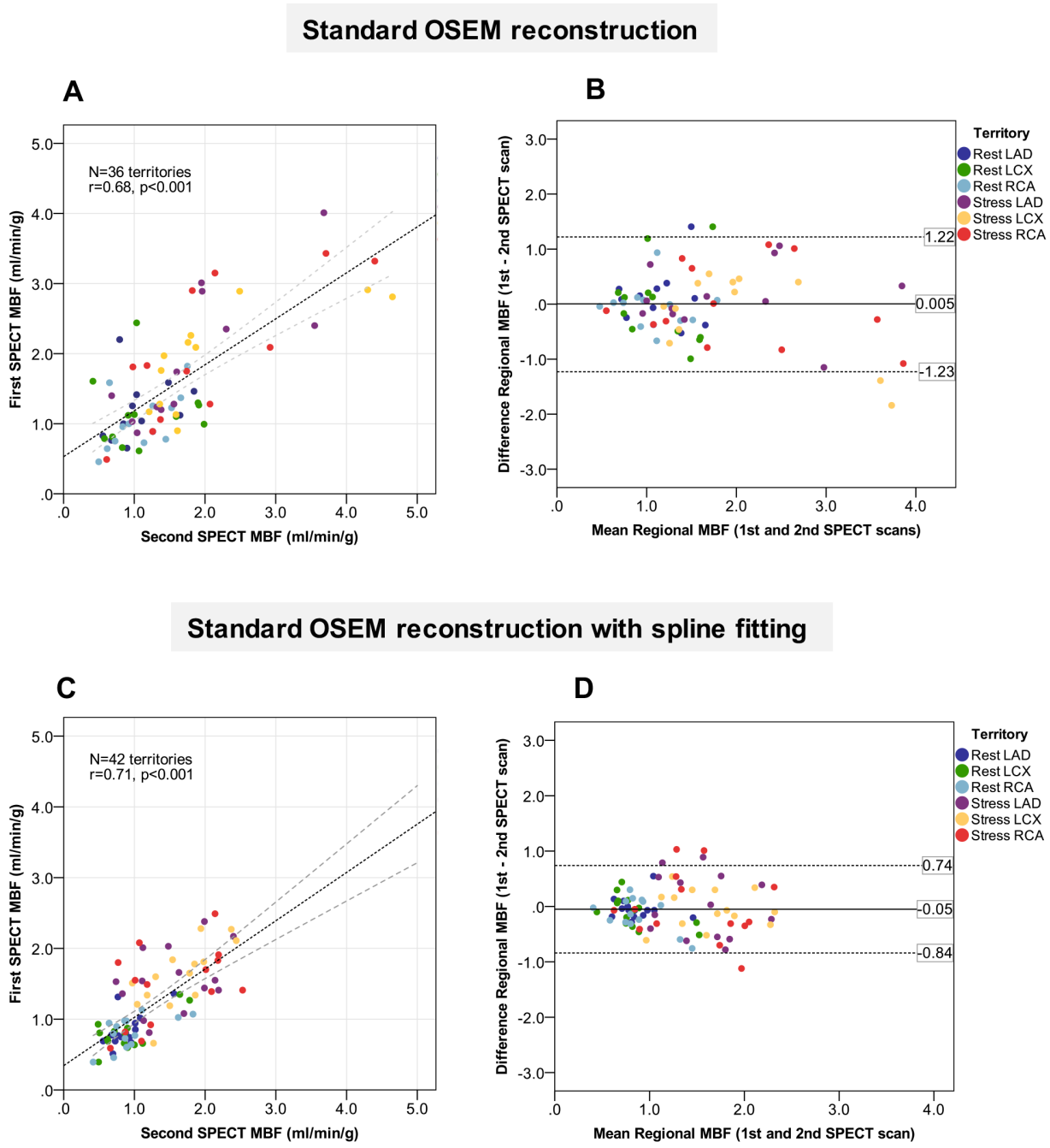

A
N=12
r=0.68, p<0.001
First SPECT scan MBF (ml/min/g)
Second SPECT scan MBF (ml/min/g)
Rest MBF
Stress MBF
B
Difference SPECT MBF (1st - 2nd scan) (ml/min/g)
Mean SPECT MBF (1st and 2nd scans) (ml/min/g)
1.11
0.04
-1.03
Rest MBF
Stress MBF
C
N=12
r=0.33, p=0.30
First SPECT scan MFR
Second SPECT scan MFR
D
Difference SPECT MFR (1st - 2nd scan)
Mean SPECT MFR (1st and 2nd scans)
1.67
-0.04
-1.76

FIGURE S7


Standard OSEM reconstruction
A
N=36 territories
r=0.68, p<0.001
First SPECT MBF (ml/min/g)
Second SPECT MBF (ml/min/g)
B
Difference Regional MBF (1st - 2nd SPECT scan)
Mean Regional MBF (1st and 2nd SPECT scans)
1.22
0.005
-1.23
Territory
Rest LAD
Rest LCX
Rest RCA
Stress LAD
Stress LCX
Stress RCA
Standard OSEM reconstruction with spline fitting
C
N=42 territories
r=0.71, p<0.001
First SPECT MBF (ml/min/g)
Second SPECT MBF (ml/min/g)
D
Difference Regional MBF (1st - 2nd SPECT scan)
Mean Regional MBF (1st and 2nd SPECT scan)
0.74
-0.05
-0.84
Territory
Rest LAD
Rest LCX
Rest RCA
Stress LAD
Stress LCX
Stress RCA

SUPPLEMENTAL FIGURE LEGENDS

FIGURE S1: Schematic summary of study cohorts

FIGURE S2: Correlation of global rest and stress coronary vascular resistance (CVR) estimated with ^{99m}Tc-sestamibi SPECT and ^{13}N-ammonia PET using standard ordered subset expectation maximization (OSEM) reconstruction without (panel A) and with spline fitting (panel B).

FIGURE S3: Sensitivity analyses of global rest and stress myocardial blood flow (MBF) and flow reserve (MFR) estimated with ^{13}N-ammonia PET and ^{99m}Tc -sestamibi SPECT using standard ordered subset expectation maximization (OSEM) reconstruction without spline fitting in 18 participants who had both reconstructions available. Correlation plots of ^{99m}Tc -sestamibi and ^{13}N-ammonia MBF (panel A) and MFR measurements (panel C). Bland–Altman plots of MBF (panel B) and MFR measurements (panel D).

FIGURE S4: Sensitivity analyses of global rest and stress myocardial blood flow (MBF) and flow reserve (MFR) estimated with ^{13}N-ammonia PET and ^{99m}Tc -sestamibi SPECT using standard ordered subset expectation maximization (OSEM) reconstruction with spline fitting in 18 participants who had both reconstructions available. Correlation plots of ^{99m}Tc -sestamibi and ^{13}N-ammonia MBF (panel A) and MFR measurements (panel C). Bland–Altman plots of MBF (panel B) and MFR measurements (panel D).

FIGURE S5: Regional rest and stress myocardial blood flow (MBF) measurements in the validation cohort using conventional ordered subset expectation maximization (OSEM) reconstruction without splines (N=30 participants/N=90 territories) (panels A and B) and OSEM with spline fitting (N=21 participants/N=63 territories) (panels C and D) reconstructions.

FIGURE S6: Reproducibility analyses of global rest and stress myocardial blood flow (MBF) and flow reserve (MFR) estimated with ^{99m}Tc-sestamibi SPECT within two weeks using standard ordered subset expectation maximization (OSEM) reconstruction without spline fitting. Correlation plots of rest and stress MBF (panel A) and MFR measurements (panel C). Bland–Altman plots of rest and stress MBF (panel B) and MFR measurements (panel D).

FIGURE S7: Regional rest and stress myocardial blood flow (MBF) measurements in the reproducibility cohort using conventional ordered subset expectation maximization (OSEM) without splines (N=12 participants/36 territories) (panels A and B) and OSEM with spline fitting (N=14 participants/42 territories) (panels C and D).

SUPPLEMENTAL TABLES

Table S1: Baseline characteristics and injected radiotracer doses in the validation groups according to available reconstructions (OSEM with and without spline fitting)

	Standard OSEM without spline fitting (N=30)	**Standard OSEM with spline fitting (N=21)**
Demographics		
Age	64.0 (54.2-72.9)	60.0 (55.5-66.1)
Male sex	16 (53.3%)	11 (52.4%)
White race	25 (83.3%)	16 (76.2%)
Non-Hispanic	28 (93.3%)	19 (90.5%)
BMI	30.7 (27.7-34.1)	30.7 (28.8-35.4)
Clinical characteristics		
History of known CAD	10 (33.3%)	5 (23.8%)
Family history of known CAD	10 (33.3%)	8 (38.1%)
Hypertension	28 (93.3%)	17 (81.0%)
Dyslipidemia	22 (73.3%)	14 (66.7%)
Diabetes mellitus	7 (23.3%)	8 (38.1%)
Current smoker	2 (6.7%)	2 (9.5%)
Previous MI or acute coronary syndrome	7 (23.3%)	4 (19%)
Revascularization history (PCI/CABG)	7 (23.3%)	4 (19%)
History of stroke or transient ischemic attack	1 (3.3%)	1 (4.8%)
Peripheral artery disease	4 (13.3%)	4 (19%)
Medications		
Beta-blockers	19 (63.3%)	11 (52.4%)
ACE inhibitors	11 (36.7%)	8 (38.1%)
ARB	7 (23.3%)	2 (9.5%)
Statins	20 (66.7%)	13 (61.9%)
Nitroglycerin	4 (13.3%)	3 (14.3%)
Antiplatelet therapy	11 (36.7%)	5 (23.8%)

Anticoagulation	6 (20.0%)	6 (28.6%)
Diuretics	12 (40.0%)	9 (42.9%)
Insulin	2 (6.7%)	2 (9.5%)
^{99m}Tc-sestamibi doses (mCi)		
Rest	7.5 ± 1.3	7.4 ± 1.2
Stress	21.5 ± 3.5	21.6 ± 3.2
^{13}N-ammonia doses (mCi)		
Rest	22.5 ± 1.4	22.6 ± 1.9
Stress	22.9 ±1.6	23.0 ±1.5

Values are presented as median and interquartile ranges (IQR) or n (%).
ACE=angiotensin-conversing enzyme; ARB=angiotensin-II receptor blocker; BMI = body mass index; CAD= coronary artery disease; CABG=coronary artery bypass grafting; PCI = percutaneous coronary intervention.

Table S2: Systemic hemodynamics and injected radiotracer doses in the study population

Validation cohort						
	SPECT (N=34)		**PET (N=34)**		**P value***	**P-value†**
	Rest	**Peak hyperemia**	**Rest**	**Peak hyperemia**		
Heart rate (beats/min)	66 (61-69)	81 (71-91)	65 (59-72)	83 (74-88)	0.58	0.64
Systolic blood pressure (mmHg)	146 (127-159)	137 (124-155)	124 (114-140)	132 (122-145)	0.001	0.23
Diastolic blood pressure (mmHg)	75 (71-83)	72 (63-80)	63 (58-69)	66 (63-72)	<0.001	0.02
Mean Arterial BP (mmHg)	100 (89-106)	94 (83-103)	85 (78-90)	87 (82-96)	<0.001	0.029
Rest dose (mCi)	7.58 ± 1.23		22.41 ± 1.67		-	
Stress dose (mCi)	21.79 ± 3.56		22.87 ± 1.58		-	
Reproducibility cohort						
	First SPECT (N=14)		**Second SPECT (N=14)**		**P-value***	**P-value†**
	Rest	**Peak hyperemia**	**Rest**	**Peak hyperemia**		
Heart rate (beats/min)	68 (59-74)	85 (75-94)	62 (59-70)	78 (70-93)	0.38	0.41
Systolic blood pressure (mmHg)	135 (117-147)	129 (113-145)	131 (115-147)	130 (108-147)	0.85	0.75
Diastolic blood pressure (mmHg)	73 (68-80)	71 (66-85)	74 (65-86)	70 (62-83)	0.88	0.53
Mean Arterial BP (mmHg)	92 (85 - 102)	95 (79-103)	93 (86-102)	91 (78-103)	0.95	0.98
Rest dose (mCi)	7.5 ± 1.64		7.4 ± 1.73		-	
Stress dose (mCi)	22.45 ± 4.96		22.46 ± 5.17		-	

*P-values refer to the comparison between rest hemodynamics obtained during SPECT and PET scans (validation cohort) and between the two consecutive SPECT scans (reproducibility cohort).

†P-values refer to the comparison between peak stress hemodynamics obtained during SPECT and PET scans (validation cohort) and between the two consecutive SPECT scans (reproducibility cohort).

Doses are expressed in milicurie (mCi)

BP: blood pressure; PET: positron emission tomography; SPECT: single photon emission computed tomography

Table S3: Mean percentage differences between global and regional cardiac-only and cardiac-and-liver retention and myocardial perfusion reserve index (MPR index) for the assessment of scatter effect from liver

Mean percentage difference between cardiac-only and cardiac-and-liver K1 and MPR index		
	Splines reconstruction	**Standard OSEM without splines**
Rest global K1	5.85±0.17	5.71±0.24
Rest LAD K1	5.74±0.18	5.62±0.25
Rest LCX K1	6.16±0.15	6.03±0.27
Rest RCA K1	6.59±0.17	6.41±0.25
Stress global K1	7.67±0.45	7.18±0.86
Stress LAD K1	7.61±0.57	7.11±1.04
Stress LCX K1	8.30±0.38	7.40±0.28
Stress RCA K1	8.32±0.71	8.24±1.50
Global MPR	1.93±0.54	1.57±0.70
LAD MPR	1.98±0.62	1.59±0.85
LCX MPR	2.28±0.40	1.46±0.50
RCA MPR	1.86±0.85	1.96±1.35

Table S4: Mean percentage differences between attenuation-corrected and non-corrected retention and myocardial perfusion reserve index (MPR index)

Mean percentage differences between attenuation-corrected and non-corrected K1 and MPR index		
	Splines reconstruction	**Standard OSEM without splines**
Rest global K1	3.62±0.10	3.53±0.15
Rest LAD K1	7.84±0.15	7.79±0.32
Rest LCX K1	1.88±0.28	1.70±0.15
Rest RCA K1	5.44±0.49	5.75±0.64
Stress global K1	4.72±0.29	4.43±0.54
Stress LAD K1	9.20±0.38	8.56±0.69
Stress LCX K1	3.91±0.33	3.27±0.16
Stress RCA K1	5.11±0.45	4.65±0.46
Global MPR	1.15±0.33	0.93±0.42
LAD MPR	1.47±0.52	0.84±0.43
LCX MPR	2.08±0.33	1.60±0.26
RCA MPR	0.65±0.23	1.04±0.85

Table S5: Comparison between regional myocardial blood flow (MBF) and myocardial flow reserve (MFR) estimates obtained with ^{13}N-ammonia PET and ^{99m}Tc-sestamibi SPECT with and without spline fitting

Standard OSEM reconstruction			
	^{99m}Tc-sestamibi SPECT (N=90 territories)	**^{13}N-ammonia PET (N=90 territories)**	**P-value**
Regional rest flow (ml/g/min)	0.87 (0.64-1.17)	0.74 (0.64-0.84)	<0.001
Regional corrected rest flow (ml/g/min)	0.96 (0.71-1.20)	0.90 (0.75-1.06)	0.091
Regional stress flow (ml/g/min)	1.87 (1.31-2.58)	1.44 (1.24-1.80)	<0.001
Regional MFR	2.24 (1.56-2.75)	1.90 (1.65-2.28)	0.025
Corrected regional MFR	1.98 (1.49-2.49)	1.61 (1.26-2.02)	0.001
Spline fitting reconstruction			
	^{99m}Tc-sestamibi SPECT (N=63 territories)	**^{13}N-ammonia PET (N=63 territories)**	**P-value**
Regional rest flow (ml/g/min)	0.62 (0.49-0.78)	0.74 (0.61-0.84)	0.005
Regional corrected rest flow (ml/g/min)	0.74 (0.58-0.91)	0.96 (0.68-1.11)	<0.001
Regional stress flow (ml/g/min)	1.29 (0.98-1.63)	1.44 (1.24-1.81)	0.009
Regional MFR	1.96 (1.63-2.48)	1.90 (1.76-2.25)	0.54
Corrected regional MFR	1.70 (1.45-2.28)	1.56 (1.21-2.03)	0.074

Myocardial blood flow (MBF) is reported in mL/min/g. Myocardial flow reserve (MFR) is calculated as the ratio between stress MBF and that at rest.
*P-values refer to the comparison between ^{99m}Tc-sestamibi SPECT and ^{13}N-ammonia groups and are based on the Wilcoxon signed-rank test.
PET: positron emission tomography; SPECT: single photon emission computed tomography; OSEM: ordered subset expectation maximization