Assessment of the Reproducibility of Baseline and Hyperemic Myocardial Blood Flow Measurements with ^{15}O-Labeled Water and PET

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PET with ^{15}O-labeled water allows noninvasive quantification of myocardial blood flow (MBF) at baseline and during pharmacologically induced hyperemia to assess the coronary vasodilator reserve (CVR = hyperemic/baseline MBF). Despite widespread use of PET, its reproducibility during one study session has not been tested. Intravenous adenosine (Ado), a powerful coronary vasodilator with a very short decay time, is commonly used for the induction of hyperemia. However, it is not known whether Ado can induce tachyphylaxis after short-term repetitive administration. In this study, we aimed to test the reproducibility of PET assessment of CVR during Ado-induced hyperemia. **Methods:** In 21 healthy volunteer men, baseline and Ado MBF were measured twice using PET with ^{15}O-labeled water to obtain two CVR assessments within 1 h. **Results:** There was no significant difference between the two baselines (0.89 ± 0.14 versus 0.99 ± 0.15 mL/min/g, mean difference 13% ± 11%) or between the two hyperemic MBFs (3.51 ± 0.45 versus 3.83 ± 0.49 mL/min/g, mean difference 10% ±14%), resulting in comparable values of CVR (4.05 ± 0.75 versus 3.93 ± 0.72, mean difference 2% ± 15%). The repeatability coefficient for MBF was 0.17 mL/min/g at baseline and 0.94 mL/min/g during hyperemia. The repeatability coefficient of the rate pressure product (RPP) was lower at baseline (1,304 mm Hg × beat/min) than during hyperemia (3,448 mm Hg × beat/min). **Conclusion:** Repeated measurements of MBF and CVR during the same study session were not significantly different, demonstrating the validity of the technique. The larger variability of hyperemic flow, as indicated by the larger repeatability coefficient, was paralleled by a greater variability of the RPP. This could mean that the greater variability of MBF during stress is more likely due to a variable response to Ado rather than to a measurement error.

Key Words: coronary circulation; myocardial blood flow; adenosine; ^{15}O-labeled water; PET

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P ET has been shown to allow noninvasive and accurate quantitative measurements of regional myocardial blood flow (MBF) if suitable tracers are used and appropriate mathematical models are applied.

Baseline and hyperemic MBF measurements allow the assessment of coronary vasodilator reserve (CVR = hyperemic/baseline MBF), an integrated parameter of endothelial function and vascular smooth muscle relaxation. PET has been widely used to assess CVR in healthy volunteers (1,2) and in patients with coronary artery disease (3), cardiovascular risk factors (4) and other cardiac diseases (5). Furthermore, measurements of CVR with PET have been used to assess the effect of pharmacological interventions such as α (6) and β blockade (7), lipid-lowering drugs (8), cardiovascular conditioning (9) and coronary angioplasty (10,11).

Comparable estimates of MBF can be obtained in normal volunteers using the ^{15}O-water and the ^{13}N-ammonia techniques (12). ^{13}N-ammonia offers an image quality superior to that of ^{15}O-water, because of its prolonged retention in myocardium and its preferential distribution into the myocardium and because of the longer half-life of the ^{13}N-isotope (9.8 min compared to 2 min for ^{15}O-water). However, the myocardial net extraction of ^{13}N-ammonia is related nonlinearly to MBF, because its first-pass tracer extraction fraction declines with increasing MBF (13). Moreover, ^{13}N-ammonia is trapped metabolically in tissue, thus raising the criticism that the uptake of ^{13}N-ammonia may be modified by factors other than blood flow (14). In contrast, the metabolically inert ^{15}O-water freely diffuses across all capillary membranes, including that of the myocardium, and rapidly equilibrates between the vascular and extravascular spaces. The first-pass extraction fraction of ^{15}O-water approaches unity and is independent of blood flow. Thus, the net extraction (as the product of the first-pass extraction and MBF) correlates linearly with MBF. The short half-life of ^{15}O allows for repetitive MBF measurements at 10-min intervals, corresponding to five half-lives. On the other hand, a traditional shortcoming of the ^{15}O-water technique includes its need for additional ^{15}O-carbon monoxide (C^{15}O) blood pool scans to define the regions of interest (ROIs) and to correct for the high ^{15}O activity in the blood pool. Because of the increased radioactive burden, the use of serial PET measurements of MBF before and after pharmacological or therapeutic interventions during the same session have met certain ethical limitations. Recently, a new technique has been developed at our institution for generating myocardial
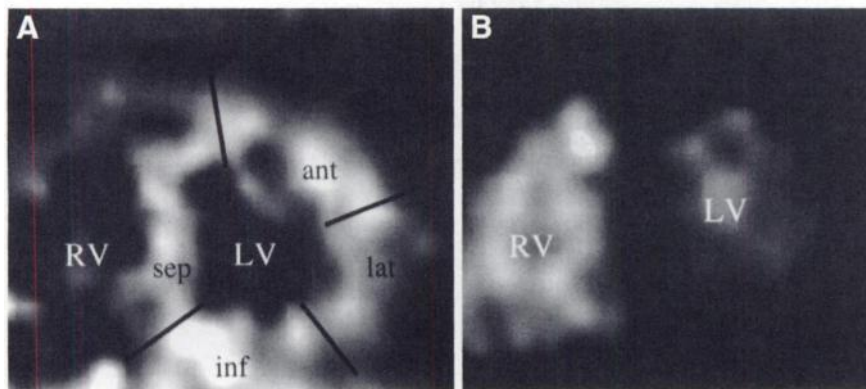
FIGURE 1. Representative midventricular short axis factor image of myocardial tissue (A) and blood pool (B) obtained with ^{15}O-labeled water. LV = left ventricle; RV = right ventricle; sep = septal; ant = anterior; lat = lateral; inf = inferior.

images directly from the dynamic ^{15}O-water scans (*15*). This development eliminates the need for additional $C^{15}O$ blood pool scans. Even though serial measurements with ^{15}O-water are possible, their reproducibility is yet to be determined. Intravenous adenosine (Ado), a powerful coronary vasodilator with a very short decay time (<10–20 s), is commonly used for the induction of hyperemia. However, it is not known whether Ado can induce tachyphylaxis after short-term repetitive administration. In this study, we tested the reproducibility of PET assessment of CVR during Ado-induced hyperemia in normal healthy volunteers, because this is the first step toward establishing this technique as a useful clinical tool.

MATERIALS AND METHODS

Twenty-one healthy volunteer men (mean age 45 ± 8 y) were studied. None of the participants had a history of cardiovascular disease or smoking. Entrance criteria included normal heart rate, blood pressure, normal resting electrocardiogram (ECG) and echocardiography, low clinical probability for coronary artery disease (*16*) and total cholesterol of ≤6.4 mmol/L (250 mg/100 mL). In addition, all volunteers were carefully instructed to refrain from caffeine intake during the 24 h before the study. A screening blood test for caffeine was also performed in each participant immediately before the PET scan. Caffeine was not detected in any participant.

The study protocol was approved by the Research Ethics Committee of Hammersmith Hospital, and radiation exposure was licensed by the United Kingdom Administration of Radioactive Substances Advisory Committee (ARSAC). All participants gave informed and written consent before the study.

Image Acquisition

Scanning was performed at the Medical Research Council Cyclotron Unit, Hammersmith Hospital, London, UK, with an ECAT 931-08/12 15-slice tomograph (CTI/Siemens, Knoxville, TN) giving a 10.5-cm axial field of view. All emission and transmission data were reconstructed using a Hanning filter with a cutoff frequency of 0.5 units of the reciprocal of the sampling interval of the projection data, resulting in an image resolution of 8.4 × 8.3 × 6.6 mm³ full width at half maximum (FWHM) at the center of the field of view (*17,18*). The optimal imaging position was determined by a 5-min rectilinear scan after the exposure of an external ^{68}Ge ring source. A 20-min transmission scan was then acquired for the purpose of attenuation correction of all subsequent emission scans. Starting after the background frame, ^{15}O-water (700–900 MBq) was injected as an intravenous bolus over 20 s at an infusion rate of 10 mL/min to measure MBF. The venous line was then flushed for another 2 min. The following acquisition frame times were used: 14 frames at 5 s, 3 frames at 10 s, 3 frames at 20 s and 4 frames at 30 s.

Image Processing

The sinograms obtained were corrected for attenuation and were reconstructed on a MicroVax II computer (Digital Equipment Corp., Marlboro, MA) using dedicated array processors and standard reconstruction algorithms. Images were transferred to a SUN SPARC 2 workstation (Sun Microsystems, Mountain View, CA) and were analyzed with MATLAB (The MathWorks Inc., Natick, MA) software packages. Myocardial images were then generated directly from the dynamic ^{15}O-water study, which eliminated the need for additional ^{15}O-carbon monoxide blood pool scans for the purpose of ROI definition. Briefly, the factor sinograms were generated by means of linear dimension reduction of the dynamic sinograms, where the required variate and covariate factors (the myocardial and blood time-activity curves) were modeled from the lung time-activity curve. The general theory for optimal linear dimension reduction of sequences of medical images has been reported (*19,20*). Factor images were generated by iterative reconstruction (*15*) (Fig. 1). ROIs were drawn within the left atrium on 4 to 6 consecutive image planes and were projected onto the dynamic ^{15}O-water images to generate blood time-activity curves (input function). Similarly, four myocardial ROIs (septal, anterior, lateral and inferior) were drawn within the left ventricular myocardium on 12 consecutive image planes and were projected onto the dynamic ^{15}O-water images to obtain tissue activity curves. Arterial and tissue activity curves were fitted to a single tissue compartment tracer kinetic model to give values of regional and global MBF (mL/min/g) as has previously been described (*3,21*).

Study Protocol

MBF was measured at baseline (baseline 1). To allow for decay of the ^{15}O radioactivity, we repeated a second MBF measurement 10-min later during adenosine-induced hyperemia (Ado 1). Adenosine was infused for 7 min at 140 µg/kg body weight/min, according to standard practice (*22*). PET acquisition was started 2 min after the beginning of adenosine infusion. After a 10-min interval, a repeat baseline MBF measurement was acquired (baseline 2), which was followed, 10 min later, by a repeat hyperemic MBF (Ado 2). CVR was calculated as the ratio of MBF during hyperemia to MBF at baseline (CVR 1 and CVR 2). In addition, because resting MBF is determined by cardiac workload (*23*), we

TABLE 1
Hemodynamics

	Baseline 1	Baseline 2	P	Ado 1	Ado 2	P
SBP	121 ± 16	121 ± 13	ns	122 ± 16	126 ± 17	ns
DBP	72 ± 11	70 ± 10	ns	68 ± 10	70 ± 10	ns
MAP	88 ± 12	87 ± 11	ns	86 ± 11	89 ± 11	ns
HR	62 ± 11	63 ± 13	ns	91 ± 15	92 ± 18	ns
RPP	7,513 ± 1,940	7,502 ± 2,066	ns	11,225 ± 2,698	11,599 ± 2,801	ns

Ado = adenosine; SBP = systolic arterial blood pressure (mm Hg); ns = not significant; DBP = diastolic arterial blood pressure (mm Hg); MAP = mean arterial blood pressure (mm Hg); HR = heart rate (beats per minute); RPP = rate pressure product (SBP × HR).
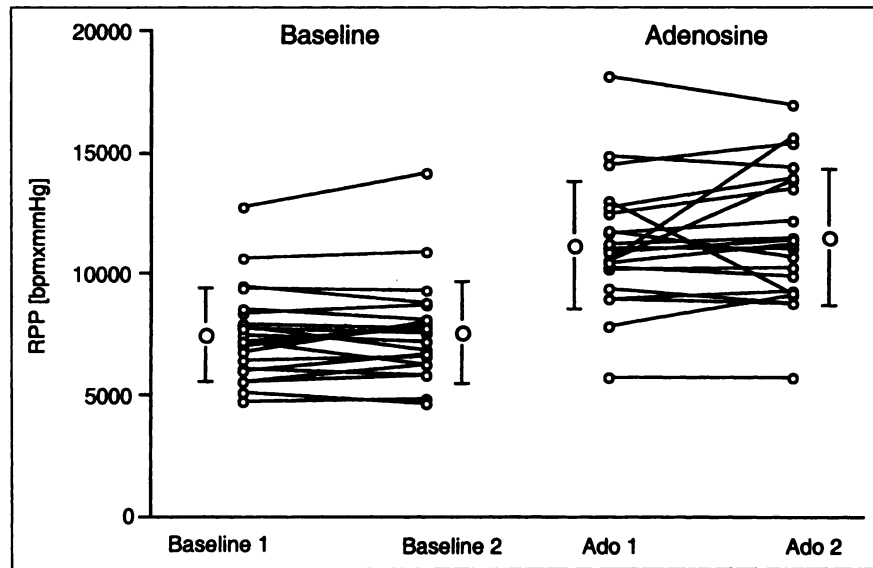

FIGURE 2. Individual values of repeated rate pressure product (RPP) measurement. RPP showed better repeatability at baseline and more variability during adenosine.

considered baseline MBF corrected (MBF_{corr}) for the rate pressure product (RPP), an index of myocardial oxygen consumption: $MBF_{corr} = (MBF/RPP) \times 10^4$ (3). Coronary resistance (Res) was calculated as the ratio of mean arterial pressure to the relative MBF. Blood pressure was recorded by an automatic cuff sphygmomanometer at 1-min intervals, the ECG was monitored continuously throughout the procedure and a 12-lead ECG was recorded at baseline and every minute during adenosine administration.

Statistical Analysis

Comparison of the hemodynamic data as well as MBF and CVR values was performed by paired Student t test. To measure the

TABLE 2
Myocardial Blood Flow

Myocardial blood flow	Baseline 1	Baseline 2	Repeatability coefficient*		Ado 1	Ado 2	Repeatability coefficient*	
			Absolute	% of mean			Absolute	% of mean
Global								
Uncorrected	0.89 ± 0.15	0.99 ± 0.15	0.17	18	3.51 ± 0.45	3.83 ± 0.49	0.90	25
Corrected	1.25 ± 0.22	1.36 ± 0.27	0.28	22				
Regional								
Septal	0.91 ± 0.16	1.02 ± 0.15	0.41	42	3.93 ± 0.78	3.63 ± 0.84	2.14	58
Anterior	0.92 ± 0.16	1.10 ± 0.20	0.46	46	3.65 ± 0.97	3.93 ± 0.78	2.03	54
Lateral	0.89 ± 0.15	0.96 ± 0.15	0.38	38	3.79 ± 0.72	3.93 ± 0.78	2.28	59
Inferior	0.89 ± 0.19	0.81 ± 0.25	0.20	22	3.52 ± 0.88	3.57 ± 0.79	1.43	41

*Repeatability coefficient equals 1.96 × SD of differences.
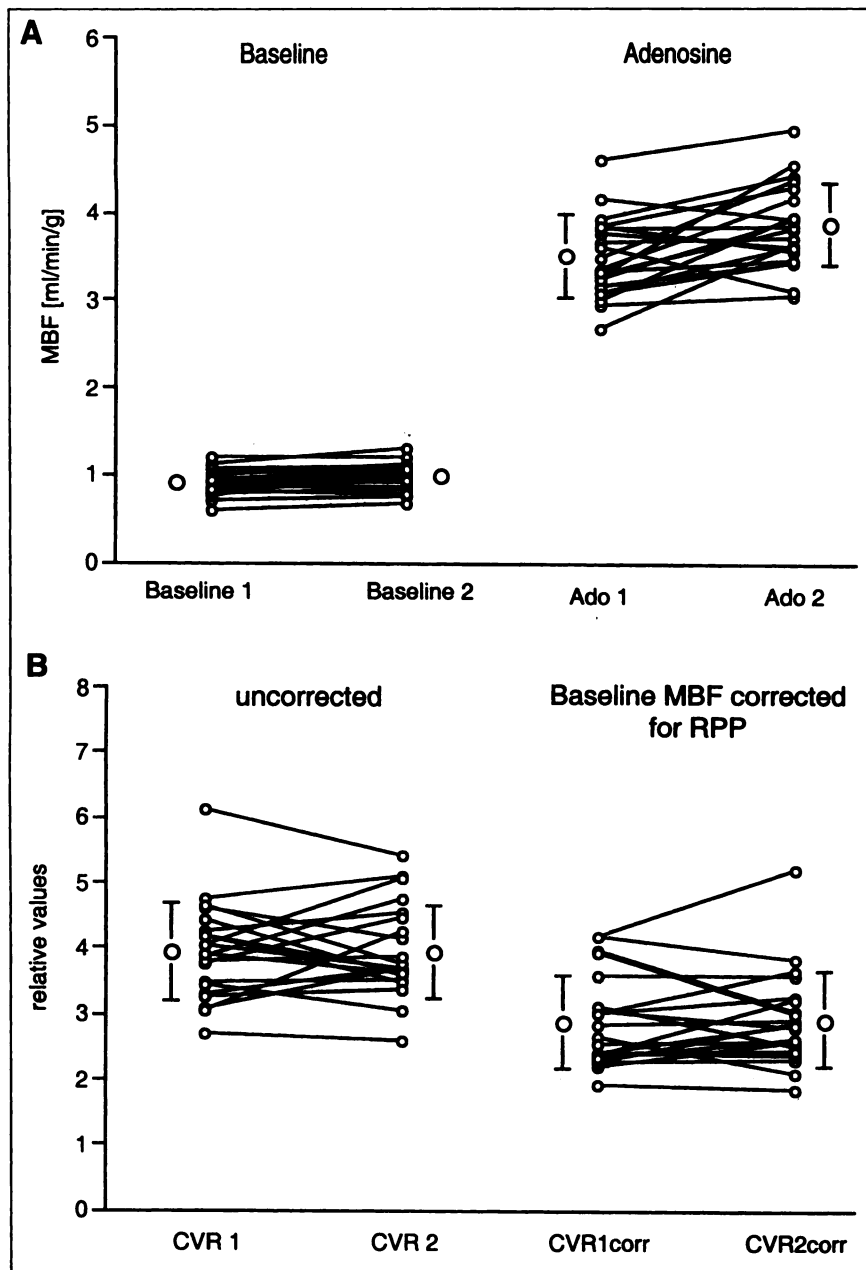Ado = adenosine.

FIGURE 3. (A) Repeated baseline and hyperemic myocardial blood flow (MBF) measurements. (B) Repeated assessment of coronary flow reserve without and with correction of baseline MBF for rate pressure product (RPP). Paired values are given for each participant. Mean values (large circles) are given as mean ± 1 SD.

precision of the PET measurement in the single study session, we calculated the repeatability coefficient, which is defined as 1.96 × SD, as proposed by Bland and Altman (24,25) and recommended by the British Standards Institution (26). Assuming that the data are normally distributed, in 95% of the cases the difference between the two measures will be less than the repeatability coefficient. In addition, the repeatability coefficient is also given as a percent of the average value of the two measurements, although the results show that the error does not change with the absolute value of the measurement.

RESULTS

All procedures were well tolerated, apart from the common side effects caused by adenosine. None of the participants had any ECG changes during the procedure.

Hemodynamics

During the two resting measurements, systolic, diastolic and mean aortic blood pressure, as well as heart rate and RPP, were almost identical (Table 1). For the values of RPP at baseline 1 and 2, we found a good repeatability (Fig. 2) with a coefficient of 1304 mm Hg × beats/min, that is, 17% of the mean RPP. The same comparison during the two adenosine infusions revealed no significant difference between the mean values (Table 1). However, the repeatability coefficient of RPP was larger during adenosine, namely 3448 mm Hg × beat/min, which equals 30% of the mean RPP.

Global Myocardial Blood Flow

The first and second baseline MBF measurements were 0.89 ± 0.15 and 0.99 ± 0.15 mL/min/g, respectively (P
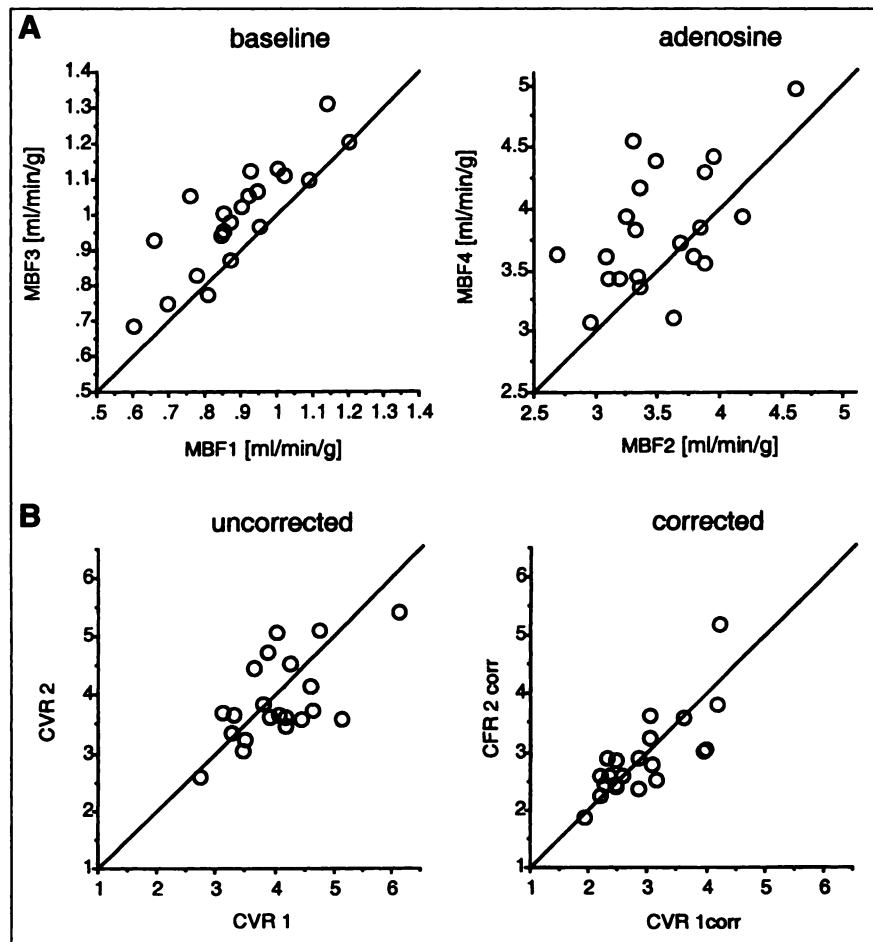
FIGURE 4. Scattergram of repeat myocardial blood flow (MBF) (A) and coronary vasodilator reserve (CVR) (B) measurements. Line of equality is shown, along which points should lie for perfect agreement.

value was not significant; Table 2), with a mean difference of 13% ± 11%. The individual whole-heart MBF values are given in Figure 3A. The agreement of both measurements is shown in Figure 4. The repeatability coefficient was 0.17 mL/min/g, equaling 18% of the mean (Fig. 5). The respective values for MBF$_{corr}$ were 1.25 ± 0.22 and 1.36 ± 0.27 mL/min/g (P value was not significant), resulting in a slightly higher repeatability coefficient of 0.20 (22% of the mean). The mean difference between both MBF$_{corr}$ was 9% ± 11%.

Adenosine induced a significant increase in mean MBF to 3.51 ± 0.45 (Ado 1, P = 0.0001 versus baseline 1) and 3.83 ± 0.49 mL/min/g (Ado 2, P = 0.0001 versus baseline 2) with no significant difference between the two hyperemic measurements (Fig. 3). The repeatability coefficient for the two hyperemic MBFs (Fig. 5) was 0.90 mL/min/g, that is, 25% of the mean.

Regional Myocardial Blood Flow

The values of the regional MBF are shown in Table 2 with their repeatability coefficients. The best (lowest) repeatability coefficient was found in the inferior wall at rest. The respective coefficients of variance are provided in Table 3.

Coronary Vasodilator Reserve

Global and regional CVR values are summarized in Table 4 and in Figure 3. CVR 1 (MBF − Ado 1/MBF − baseline 1;

relative values) was 4.05 ± 0.75 and CVR 2 (Ado 2/baseline 2) was 3.93 ± 0.72 (P value was not significant), resulting in a mean difference of 1.7% ± 16%. After correcting the baseline MBF for RPP, we found the respective CVR values to be 2.90 ± 0.71 and 2.91 ± 0.72 (P value was not significant), with a mean difference of 2.0% ± 15%. The repeatability coefficient was 1.32 (33% of the mean) for CVR and 0.98 (34% of the mean) for corrected CVR (CVR$_{corr}$) (Fig. 6). Thus, the agreement was not increased by correcting MBF baseline for RPP. The regional CVR values showed a considerably lower repeatability than the global ones.

Coronary resistance did not differ significantly between the two resting (99 ± 22 versus 89 ± 17 mm Hg/mL/min/g; P was not significant) and the two hyperemic (25 ± 4 versus 23 ± 4; P was not significant) study conditions.

DISCUSSION

This study was designed to determine the reproducibility of MBF measurements with PET. This is an important issue, because PET is increasingly used in studies that compare the effect of various interventions. At the same time, PET's short-term reproducibility during a single study session has not yet been fully documented. A study to determine the
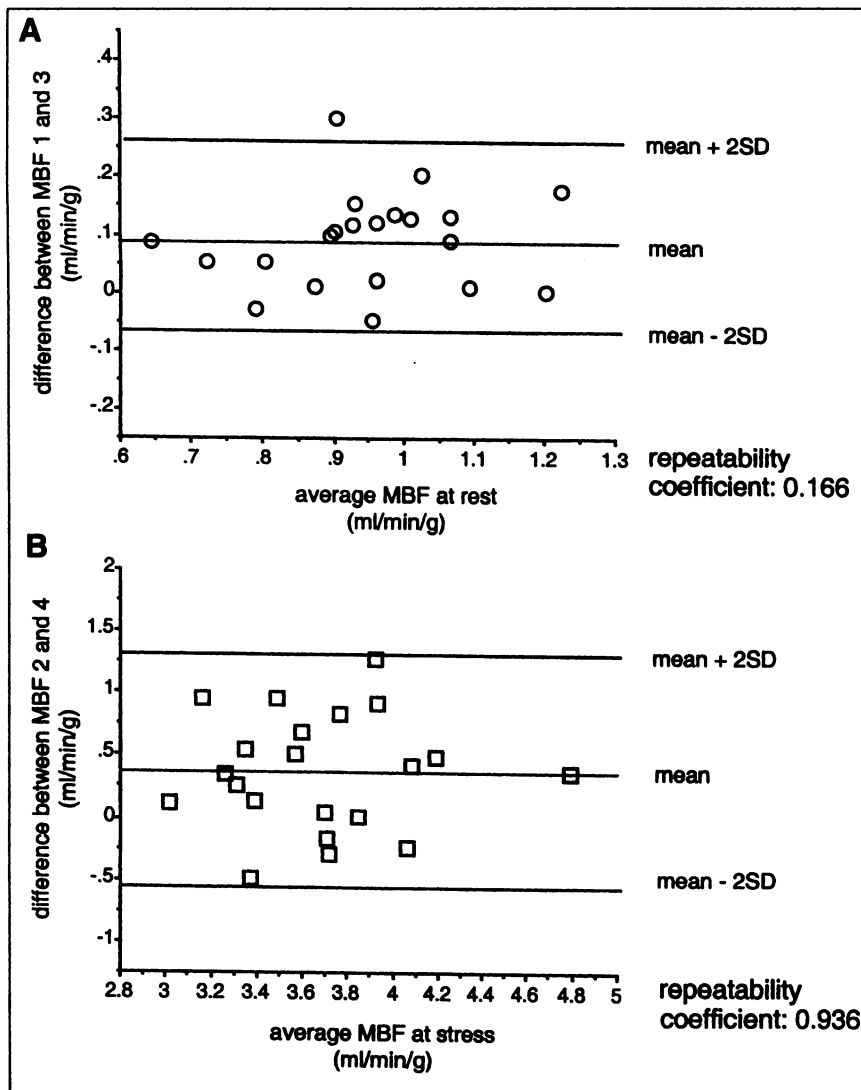
FIGURE 5. Repeatability of myocardial blood flow (MBF) measurements. The differences of two resting (A) and hyperemic (B) MBF values are plotted against their average value. Reproducibility is better at baseline.

reproducibility of a technique for MBF assessment should be performed within a well-defined, short period of time to avoid confounding factors such as seasonal, day-to-day and circadian variations of MBF, hemodynamic changes and the effects of smoking and of certain foods and beverages. Because of the potential effects of certain underlying diseases and their treatments on cardiac function and flow, we have included only carefully selected normal healthy

volunteers. The short half-lives of ^{15}O-water (2 min) and adenosine (10–20 s) allow for repeated measurements of baseline and hyperemic MBF and CVR during the same study session (within 1 h), thus overcoming the specified limitations.

TABLE 3
Myocardial Blood Flow: Coefficient of Variance

Myocardial blood flow	Baseline 1	Baseline 2	Ado 1	Ado 2
Global	16	16	13	12
Septal	20	24	16	20
Anterior	17	27	17	20
Lateral	18	20	16	20
Inferior	22	23	24	22

Ado = adenosine.

TABLE 4
Coronary Vasodilator Reserve Measurements

CVR	CVR 1	CVR 2	Repeatability coefficient Absolute	Repeatability coefficient % of mean
Global				
Uncorrected	4.05 ± 0.75	3.93 ± 0.72	1.32	33
Corrected	2.90 ± 0.70	2.95 ± 0.72	0.98	34
Regional				
Septal	4.01 ± 1.14	3.84 ± 1.04	2.89	73
Anterior	4.07 ± 1.30	3.65 ± 0.84	3.16	82
Lateral	4.33 ± 1.25	3.67 ± 0.73	2.86	72
Inferior	3.98 ± 1.14	4.25 ± 1.36	2.80	68

*Repeatability coefficient equals 1.96 × SD of difference.
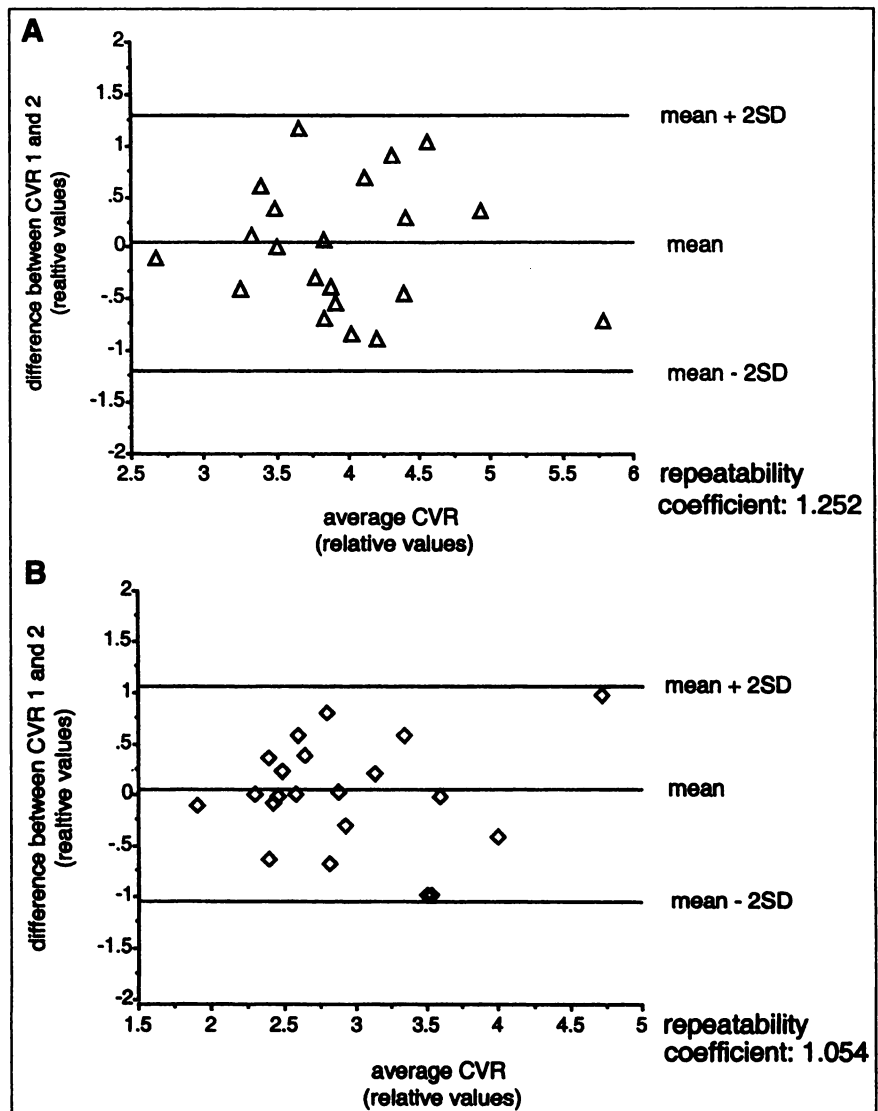CVR = coronary vasodilator reserve.

FIGURE 6. Repeatability of coronary vasodilator reserve (CVR). Differences of CVR (A) and CVR$_{corr}$ (B) values are plotted against their average value. There is no improvement in reproducibility using CVR$_{corr}$.

Reproducibility of Blood Flow at Rest

Global Myocardial Blood Flow. For the measurement of global MBF at baseline, we found a repeatability coefficient of 0.17 mL/min/g (equaling 18% of the mean global MBF at baseline), which indicated high reproducibility of the method. This measure of repeatability accounts for both methodological error and any physiological variability. MBF at baseline largely depends on cardiac work and myocardial oxygen consumption. Therefore, MBF at baseline would be expected to vary with the RPP. To allow meaningful interpretation of the quantitative data, it has been proposed that baseline MBF should be corrected for RPP, an index of external cardiac work (*3,27*). However, in our study, the correction of MBF for RPP did not improve the agreement between the first and the second measurements, because there was only a small variability of RPP between rest 1 and 2. The correction of resting MBF for RPP is probably more important when different populations are compared. The low variability of RPP at rest 1 and 2 underlines the rapid

recovery of the hemodynamic conditions after adenosine stress and, thus, the reproducibility of our data.

Regional Myocardial Blood Flow. Regional resting MBF was similar in the four left ventricular segments with a comparable coefficient of variance in all regions. However, compared to the global MBF values, the agreement between these two measurements in each segment was considerably smaller. This could be due in part to methodological reasons, because ROIs with a smaller size have poorer count statistics compared to ROIs with a larger size. However, in this setting, it seems important to consider that spatial heterogeneity of myocardial perfusion is a well-accepted fact (*28*) and may have influenced our results. Regional variability of perfusion has been observed in the hearts of all species studied thus far (*29–36*). King et al. (*37*), for example, have found that even after correcting for methodological error and temporal variability, flow to small regions of the left ventricle of conscious baboons ranged almost 6-fold in its

extremes. There is still not a definite explanation for such heterogeneity. Differences in local metabolic needs, perhaps secondary to differences in regional function, have been suggested (35,37). Temporal heterogeneities may cause changing variations between regions (38). Spatial heterogeneity in regional MBF has been reported to be linked to arteriolar or tissue oxygen partial pressure over a broad range, from 40–200 mm Hg in dogs (39). Furthermore, a linear relationship between coronary flow distribution and tissue norepinephrine content may exist (40). Thus, physiologic heterogeneity may account for the larger variability in segmental MBF, and temporal variations may explain, at least in part, the fact that regional MBF measurements are less reproducible than global MBF.

Reproducibility of Hyperemic Blood Flow

The two hyperemic measurements of global MBF revealed a repeatability coefficient of 0.94 (25% of the mean value), which was slightly higher than that observed for resting MBF. Similarly, for all left ventricular segments, regional MBF showed a higher repeatability coefficient, indicating a reduced agreement compared to the resting global MBF. The reduced repeatability of hyperemic global and regional MBF may in part be explained by the variability of the response to adenosine. In fact, the RPP showed a considerably greater repeatability coefficient (3448 mm Hg $\times$ beats/min or 30% of mean value) in response to adenosine than that found for the two resting conditions. The mean difference of 373 (Fig. 2) indicates an insignificant shift of RPP toward higher values during Ado 2, thus excluding the possibility of any tachyphylaxis to adenosine.

Because MBF during maximal vasodilation is mainly dependent on coronary driving pressure (i.e., mean coronary artery pressure), we have estimated the coronary resistance during adenosine. In fact, resistance was almost identical during both hyperemic MBF measurements (24 $\pm$ 4 versus 23 $\pm$ 4 mm Hg/mL /min/g; P value was not significant).

As a result of the reproducibility of global MBF measurements at rest and during adenosine, we found similar values for CVR 1 (3.96 $\pm$ 0.75) and 2 (3.97 $\pm$ 0.71), with a coefficient of variance of 19 and 18, respectively. However, the repeatability coefficient was 1.252 (32%), which again reflects the variability of the hemodynamic response to adenosine. Correction of resting MBF did not improve the repeatability of CVR, which suggests that such correction is not mandatory for within-group comparison when hemodynamic parameters such as RPP remain stable.

Studies of regional blood flow have demonstrated that the distribution of maximal flow—as with resting flow—is extremely heterogeneous (33), resulting in a wide dispersion of coronary reserve (28). Seemingly intrinsic properties, such as maximal regional blood flow, vary significantly throughout the heart and over time. Thus, the modest repeatability of regional CVR in our study may be mainly explained by physiological heterogeneity rather than by methodological issues.

Repetitive MBF measurements in the same patients have been reported by others, albeit usually when comparing estimates of flow using two different approaches (12). Nagamachi and coworkers (27) have reported significant reproducibility of their MBF measurements, but they did not provide a repeatability coefficient for their data obtained on different days. By using ^{13}N-labeled ammonia and dipyridamole, these authors precluded repeated measurements on the same day due to the relative long half-lives of these substances. To the best of our knowledge, our study is the first to report on the short-term reproducibility of MBF using PET and ^{15}O-labeled water and to show the range of repeatability. How far apart measurements can be without causing a problem will be a question of judgment for each individual study and clinical setting. Future studies should include participants with different cardiovascular pathologies to determine the value of this technique as reliable clinical tool. Our data provide useful information for choosing the sample size of future clinical PET studies.

CONCLUSION

We have demonstrated the short-term reproducibility of noninvasively derived quantitative MBF measurements with ^{15}O-water PET. Our results support the use of PET as the optimal technique for the noninvasive determination of MBF after intervention (either pharmacological or mechanical) on the coronary circulation. The reproducibility of PET in patients with heart disease remains to be determined.

ACKNOWLEDGMENT

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