Calf Flow Reserve with $H_2{}^{15}O$ PET as a Quantifiable Index of Lower Extremity Flow

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Objective measures of recruitable blood flow are of importance in angiogenesis trials. We validated a new PET-derived flow reserve (FR) measurement in healthy subjects and subjects with peripheral artery disease (PAD). **Methods:** Five healthy volunteers and 5 subjects with PAD underwent cannulation of the femoral artery and vein. Basal and maximal flow (100 μg/kg/min of adenosine infused intraarterially) in the lower extremity was determined using thermodilution (TD) techniques. Subjects then underwent plethysmography (PL) followed by PET measurements of blood flow at the calf level. For the PET studies, a transmission scan followed by injection of 1.85 GBq (50 mCi) $H_2{}^{15}O$ and dynamic scanning for 5 min were acquired in five 1-min frames. Regions of interest were drawn on successive PET image slices, and radioactivity was quantified from the first-minute scan after injection. FR for each of the 3 modalities was expressed as the ratio of adenosine to basal flow. **Results:** PET-derived FR correlated strongly with TD ($r = 0.82$; $P = 0.004$) but not with PL ($r = 0.17$; $P = 0.85$). The mean average difference in FR between healthy volunteers and PAD subjects was 13.0 with PET and 4.5 with TD. The intra- and intersubject variability for PET expressed as the coefficient of variation was 10.5% and 29.0% for healthy subjects and 7.0% and 52.9% in PAD, respectively. **Conclusion:** As expected, FR was significantly lower in PAD subjects compared with healthy subjects as assessed with TD and PET but not with PL. PET-derived FR appears to be reproducible and generates sharper and higher indices of recruitable flow in healthy subjects and PAD. These findings have implications for the use of PET-derived FR as a sensitive index of recruitable flow in angiogenesis trials.

Key Words: PET; flow reserve; thermodilution; angiogenesis; peripheral artery disease

J Nucl Med 2003; 44:915–919

A reliable estimate of maximal recruitable flow would be of considerable benefit in the management of subjects with peripheral artery disease (PAD). Resting blood flow is often preserved in all but the most advanced subjects with ongoing rest pain and tissue loss. Provocative tests such as exercise testing provide semiquantitative information at best, which may not be relevant in a subset of subjects unable to perform exercise. Another issue of potential clinical relevance is that, in subjects with PAD, collateral vessels of <500 μm may significantly contribute to blood flow and their influence may be hard to assess by conventional means (*1*). A simple, reproducible technique that accurately measures changes in flow in response to a maximal vasodilator stimulus may help quantify individual vasodilator responses and may be useful to assess severity of disease and response to therapy such as angiogenic growth factors. The use of flow reserve (FR), as opposed to absolute flow, provides a useful estimate because it circumvents variability due to factors influencing basal flow and avoids interindividual differences secondary to technique and anatomic variations in the arterial circulation (*1*,*2*). PET-derived FR has been used extensively in the coronary circulation (*3*). Recent refinements in reconstruction methods of PET images have enabled investigators to accurately perform pixel-by-pixel quantitation of regional muscle blood flow in the lower extremities of healthy subjects and subjects with PAD (*4–10*). In this study, we compared $H_2{}^{15}O$ PET-derived calf FR as an index of lower extremity limb perfusion in healthy subjects and subjects with PAD and compared this technique with calf plethysmography (PL)- and thermodilution (TD)-derived measures of limb perfusion.

MATERIALS AND METHODS

The institutional review board and the clinical research center at the University of Michigan approved the protocol. All subjects provided complete informed consent before participation. Baseline blood chemistries were drawn at a screening visit before the actual study date to assess eligibility. Control subjects were healthy volunteers recruited through advertisements placed on the bulletin boards of the hospital and were accepted as participants provided they did not have the following: (a) hypertension (>140/80 mm Hg); (b) hyperlipidemia (total cholesterol of >240 mg/dL); (c) diabetes (fasting glucose of >100 mg/dL or glycosylated hemoglobin of >8.0); (d) current smoking; (e) history of asthma or chronic obstructive pulmonary disease (due to possible bronchospasm with adenosine); (f) any form of atrioventricular block; (g)

Received Jul. 29, 2002; revision accepted Feb. 20, 2003.

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known vascular disease in the past, including cerebrovascular accident, myocardial infarction (clinical or electrocardiographically based), or lower extremity vascular disease; and (h) women who were pregnant or desired to become pregnant or were currently breast feeding. Subjects with PAD were required to have a resting ankle brachial index of <0.8 or angiographic evidence of atherosclerotic disease of the superficial femoral or popliteal artery or the infrapopliteal arteries with ≥ 1 run-off vessel. Individuals with aortoiliac or common femoral disease were excluded. Subjects were admitted either the morning of or the evening before the study. All vasoactive medications were withheld for at least 12 h before the study, and all long-acting vasoactive medications were withheld for at least 24 h. In 6 subjects, TD measurements were performed first, followed by PL and, subsequently, PET. This sequence was arbitrarily varied in subjects to reduce bias.

Femoral Artery Instrumentation

All studies were performed in the supine position after the subject had emptied his or her bladder. The groin area was prepared using sterile techniques. A catheter was inserted into the antecubital vein for infusion of substances. Subsequently, the femoral artery and vein were cannulated. A 5-French sheath (Cordis Laboratories, Inc.) was placed in the femoral vein to allow the insertion of a custom-designed 5-French double-lumen TD catheter (Baxter Scientific, Edwards Division) to measure leg blood flow as described (*11,12*). The right femoral artery was cannulated with a 5-French sheath (Arrow International) to allow simultaneous infusion of drugs through a 4.0-French sheath inserted through the central lumen. Heart rate and blood pressure were monitored continuously via precordial leads and a pressure transducer connected to a vital signs monitor.

Lower Extremity Blood Flow by TD

After a 15-min equilibration period, baseline blood flow was determined with saline infusion (to match the volume and the rate of subsequent infusion with adenosine) into the femoral artery. Four minutes into the infusion, 10 TD measurements were obtained at the rate of 1 measurement every 30–45 s. During each of these measurements, 1 mL of ice-cold normal saline was injected into the femoral vein via the TD catheter. The resulting TD curves were recorded and visually inspected for integrity. A computer that integrates the area under the TD curve and displays the flow rate (in L/min) was used. These values were averaged to yield the resting flow. After this, subjects underwent intrafemoral artery infusions of adenosine (Adenoscan; Fujisawa) at the rate of 100 μg/kg/min (mean dose, 50 mg) using a programmable pump. As before, 10 TD measurements were obtained at the rate of 1 measurement every 30–45 s. These values were averaged to yield the flow in response to adenosine. Average lower extremity blood flow with adenosine was expressed against each patient's average baseline flow with the infusion of saline to yield the FR. Additional measurements with TD were obtained before PL and before PET (after allowing a 10-min washout for the drug) to ensure restoration of flow to baseline values before embarking on a second modality.

Calf PL for Calf Blood Flow

A mercury-filled Silastic (Dow Corning Corp.) strain gauge was placed in the widest part of the calf. The strain gauge was connected to a plethysmograph (model EC-4; DE Hokanson) calibrated to measure the percentage change in volume and connected to a chart recorder to record the flow measurements. For each measurement, a cuff placed around the thigh was inflated to 40 mm Hg with a rapid cuff inflator (model E-10; DE Hokanson) to occlude venous outflow from the extremity. Basal measurements were obtained by infusing normal saline similar to the TD protocol every 15 s (7 s for each measurement); 7 or 8 readings were obtained for each mean value. Calf blood flow (CBF) in response to adenosine was measured 4 min into the infusion and expressed in L/mg/min. Calf FR is the ratio of adenosine-induced flow in the calf and basal flow.

Adenosine Infusions

The infusion pump (model AS50; Baxter) was set to 100 μg/kg/min, and the infusions were for 10 min each. This corresponds to a cumulative dose of 70 mg per infusion. Previous studies have shown that this dosage of adenosine is adequate to induce maximal peripheral vasodilatation (*13,14*).

PET

Production of $H_2{}^{15}O$. A TCC CS-30 26-MeV proton cyclotron (The Cyclotron Corp.) was used for $H_2{}^{15}O$ (half-life, 123 s) production by proton irradiation of $H_2{}^{16}O$ ([p,pn] reaction).

Scanning Protocol. An 8-ring ECAT 931/08 tomograph (Siemens/CTI Corp.) was used for scanning. The scanner has an axial resolution of 6.7 mm and an intrinsic in-plane resolution of 6.5 mm. The subjects were positioned in the tomograph in a supine position with the calf region within the gantry and fixed on the bed by a leg-holding band. Before emission scanning, a transmission scan for correction of photon attenuation was performed for 15 min with a removable ring source containing ^{68}Ge. For the blood flow studies, 1.85 GBq (50 mCi) $H_2{}^{15}O$ were injected intravenously (10-mL volume infused over 30 s using an automated injector) and a dynamic scan was performed for 5 min using 5 frames, each 60 s in duration. PET acquisition began approximately 10 s after arrival of radioactivity in the field of view. This was done to minimize effects of blood-borne radioactivity in the field of view. Four such blood flow PET acquisitions were performed (time between injections, approximately 14 min): 2 baseline studies and 2 studies after adenosine infusion. The concentration of radioactivity in blood was monitored at 2 and 5 min after the beginning of the scan. Total injection dose, total blood volume of arterial sampling, and total scanner time for $H_2{}^{15}O$ PET studies per series were 7.4 GBq (200 mCi), 8 mL, and 70 min, respectively. Dead-time and random coincidence events were corrected in each frame separately, and correction for photon attenuation was done with data obtained from the transmission scan. Dynamic datasets were reconstructed to a 128×128 matrix using standard filtered backprojection and a Hanning filter with a cutoff value of 0.4 (80% of the Nyquist frequency).

Estimation of PET FR. FR was estimated from the initial 1-min PET scan. Data acquired from the first 40–120 s (60-s data) after radiotracer arrival have been shown to yield a good estimate of relative blood flow (*15,16*). The concentration of $H_2{}^{15}O$ tracer in tissue, C(t), was measured in calf muscle from regions of interest (ROIs) drawn on successive PET image slices containing calf muscle at baseline and in response to adenosine. The region was drawn on the average image over all scans and, thus, the same ROI was used in both baseline and adenosine scans. This region was drawn in an attempt to include as much muscle as possible, while avoiding bone and major arteries. Thus, the ROI used varied slightly from subject to subject.

We looked at longer intervals (out to the full 5 min), and, as expected, the radiotracer distribution showed changes from a flow

distribution toward that of water distribution volume. Calf FR was defined as the ratio of adenosine-induced flow in the calf and basal flow.

Statistical Analysis

All values are expressed as mean ± SEM unless otherwise stated. Comparison of continuous variables was performed using unpaired *t* tests and a P value < 0.05 was considered significant. Spearman correlation coefficients and significance were performed to assess the relationship between the various measures. All statistical analyses were performed using GraphPad Prism software (version 3.02; GraphPad Software, Inc.).

RESULTS

The mean age of the PAD subject population ($n = 5$) was 60 ± 8 y and was substantially older than the healthy volunteer population ($n = 5$; 33 ± 16 y; $P < 0.01$ by unpaired *t* test). As expected, the PAD population smoked (60% vs. 0% in healthy volunteers) and was marginally hypertensive (95 ± 9 vs. 77 ± 7 mm Hg; $P = 0.007$) with a trend toward higher concentrations of triglycerides (177 ± 77 vs. 118 ± 91 mg/dL; $P = 0.3$) and lower high-density lipoprotein cholesterols (56 ± 23 vs. 47 ± 18 mg/dL; $P = 0.5$). Total and low-density lipoprotein cholesterol concentrations were comparable (190 ± 30 vs. 167 ± 23 mg/dL and 99 ± 18 vs. 91 ± 12 mg/dL, respectively). Mean ankle brachial index in the PAD subjects was 0.81 ± 0.3, and all subjects had calf claudication secondary to infrainguinal disease. The mean flow rate by TD in healthy volunteers (in the entire lower extremity) was 0.58 ± 0.49 L/min at rest, increasing to 3.93 ± 1.9 L/min with adenosine, representing a 9-fold increase. In PAD subjects, the mean flow rates at rest were significantly lower at 0.24 ± 0.07 L/min ($P < 0.01$ vs. healthy volunteers by unpaired *t* test) and increased 5-fold to 1.01 ± 0.3 L/min with adenosine ($P < 0.01$ vs. adenosine flow in healthy volunteers). Calf flow by calf PL in healthy volunteers measured 0.21 ± 0.89 mL/dL/min and increased to 0.45 ± 0.20 mL/dL/min with adenosine. In PAD subjects, basal flows by PL were 0.24 ± 0.71 mL/dL/min and increased to 0.85 ± 0.38 mL/dL/min, a 3.5-fold increase. PET determinations of CBF in healthy volunteers, as quantified by the GBq/mL (mCi/mL) normalized to a 1.85-GBq (50 mCi) injection, revealed a value of 0.004 ± 0.001 at rest that increased to 0.066 ± 0.011 with adenosine. In PAD subjects, flow was 0.004 ± 0.001 at rest, increasing to 0.027 ± 0.013. The FR was calculated for each of the 3 techniques by dividing adenosine-induced changes in blood flow by baseline blood flow. The FR for each subject in the study derived in this manner is enumerated in Table 1. PET resulted in consistently higher FR measurements in all subjects compared with PL and in all but 2 individuals with TD (both with PAD), where it yielded comparative flow ratios. Figure 1 demonstrates the PET uptake with $H_2{}^{15}O$ in the calf region of a healthy volunteer and a subject with PAD. At baseline, there is very little activity in both the healthy volunteer and the PAD subject in the right calf. In response to adenosine, there is a marked increase in activity in the healthy volunteers, whereas in PAD this response is attenuated. The mean FR with PET was higher than with TD and PL for healthy volunteers and for PAD subjects (Fig. 2). The mean FR in the PAD subjects was attenuated, when compared with the control subjects (Fig. 2) with both PET (17.8 ± 5.1 vs. 6.7 ± 3.5; $P \leq 0.001$ by unpaired *t* test) and TD (8.9 ± 3.9 vs. 4.5 ± 1.7; $P \leq 0.05$ by unpaired *t* test) but not with PL (2.3 ± 0.7 vs. 3.4 ± 0.8; P = not significant). There was a strong correlation between TD measurements and PET measurements for all subjects ($r = 0.82$; $P = 0.004$; Fig. 3). Inter- (between subject) and intrasubject variability with PET was assessed by the coefficient of variance. Intersubject variability was 29.0% ± 5.2% and 52.9% ± 3.5% for healthy volunteers

TABLE 1
Lower Extremity Blood Flow

	TD (L/min)			PL (mL/dL/min)			PET (GBq/mL)		
Subject no.	Base	Ad	FR	Base	Ad	FR	Base	Ad	FR
Healthy volunteers									
1	0.12	1.6	13.5	2.62	5.14	1.96	0.003	0.063	0.807
2	1.39	6.8	4.9	3.9	9.77	2.51	0.005	0.060	0.453
3	0.37	4.6	12.5	5.89	11.63	1.97	0.003	0.081	0.905
4	0.64	3.7	5.6	2.39	3.69	1.54	0.005	0.073	0.541
5	0.39	3.0	7.7	2.59	8.81	3.4	0.003	0.053	0.578
PAD subjects									
6	0.2	1.4	6.8	2.53	9.84	3.89	0.006	0.036	0.212
7	0.2	0.9	4.9	5.43	24.06	4.43	0.004	0.013	0.124
8	0.34	0.75	2.2	4.7	11.28	2.4	0.004	0.022	0.224
9	0.17	0.69	4.1	3.2	9.01	2.82	0.003	0.019	0.206
10	0.29	1.24	4.3	4.5	16.67	3.7	0.003	0.043	0.470

Baseline (Base) and adenosine (Ad)-stimulated measurements of lower extremity blood flow as assessed by 3 different modalities in healthy volunteers and subjects with PAD. FR was calculated as ratio of Ad-to-Base flow.

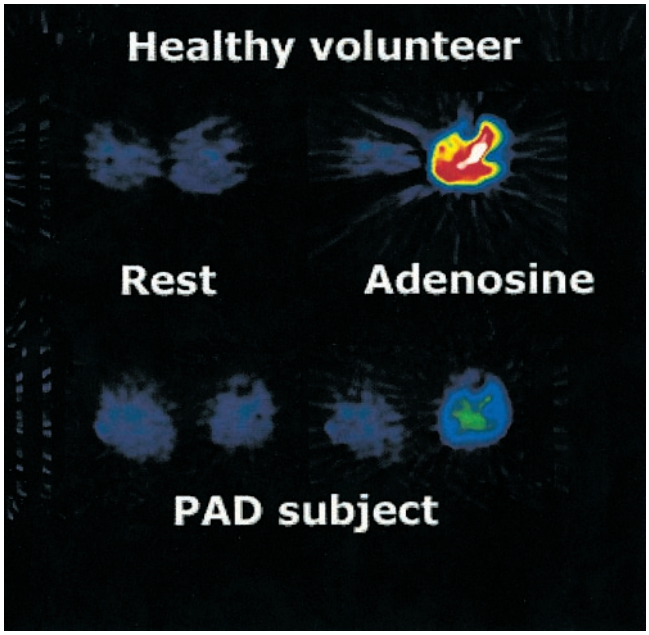

FIGURE 1. Example of PET images in healthy volunteer and subject with PAD. Adenosine was selectively infused into left lower extremity. Both resting images and images after adenosine administration are shown.

and PAD subjects, respectively. Similarly, intrasubject variability was 10.5% ± 0.1% and 7.0% ± 0.06% for control and PAD subjects, respectively. The mean absolute differences in FR between PET compared with PL and PET compared with TD were 5.55 ± 4.6 and 9.35 ± 7.8, respectively, for all subjects.

DISCUSSION

The main finding of the study is that determination of adenosine vasodilator reserve at the calf with $H_2{}^{15}O$ PET appears to generate better resolution of recruitable flow in the calf compared with 2 conventional techniques used to evaluate flow.

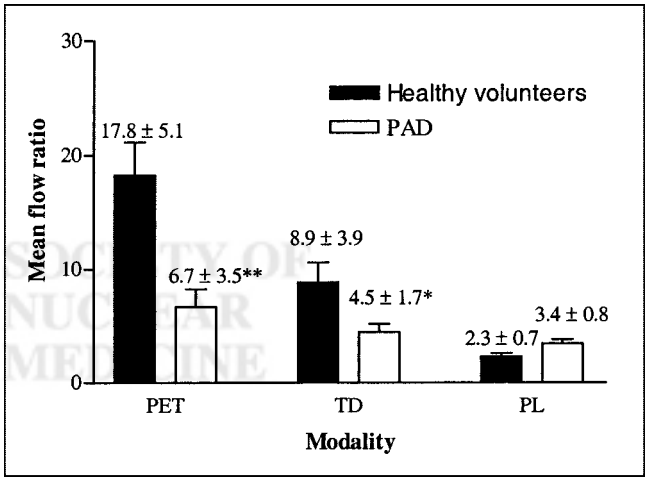

FIGURE 2. Mean flow ratios of healthy volunteers and subjects with PAD using all 3 modalities. $^{**}P \leq 0.001$; $^{*}P \leq 0.05$.

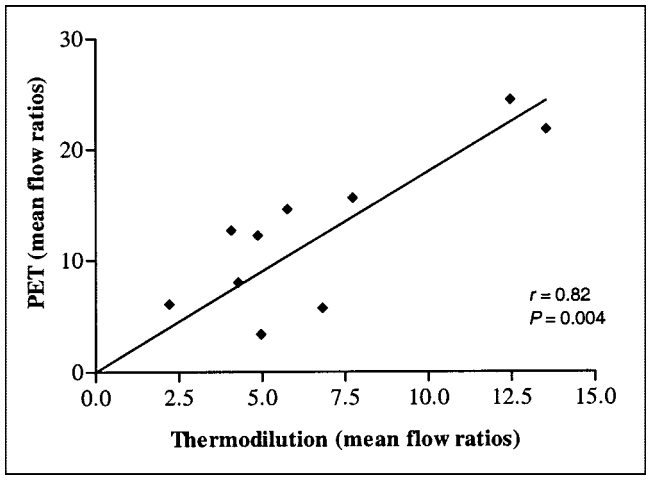

FIGURE 3. Correlation of mean flow ratio of all 10 subjects using TD and PET.

As expected, mean flow rates in the PAD subjects as measured by a gold-standard technique (TD) were significantly attenuated compared with healthy volunteer subjects. Flow reserve in the lower extremity was correspondingly attenuated in PAD with TD. Because it was not possible to arrive at a measurement that would yield total lower extremity blood flow akin to TD with either PL or PET, we had to choose a portion of the lower extremity for these alternate modalities. We chose the calf region for measurements with PL and PET because of the fact that it is the most commonly referred site of claudication in subjects with PAD in general, and specifically in individuals with infrainguinal disease, who comprised the population with PAD in this study (*17*). PL picked up changes in CBF in response to adenosine but was not helpful in discriminating subjects with PAD from healthy volunteers. In most cases (8/10), PET resulted in better discriminative ability than TD because the magnitude of differences seen with PET in PAD was higher in most subjects than that using other techniques. The only exception to this appeared to be in subjects with lower mean flow ratios (in PAD), where PET and TD appeared to be comparable.

The concept of FR as an index of regional perfusion is not new (*18*). It has been extensively applied in the coronary circulation, where alterations in myocardial flow ratios (coronary FR) have been demonstrated to be attenuated with a variety of risk factors and have been demonstrated to be improved with pharmacologic strategies such as lipid lowering and L-arginine (*19–22*). With the emergence of angiogenic pharmacotherapies for the treatment of PAD, the availability of a quantifiable index may make it feasible to assess responses to therapy in situations where alternate measurement of flow or angiographic assessment are either difficult or impractical (*23*). Although our study involved invasive cannulation of the femoral artery to provide vali-

dation with TD, this modality could be easily modified to a noninvasive assessment with intravenous adenosine (140 μg/min) that is well known to cause systemic vasodilatation and is used routinely in adenosine stress studies (*24*). These findings justify the development of a simple PET-derived noninvasive assessment of FR as an index of disease severity and response to therapy.

The major limitation of this study is the usage of TD flow measurements in the entire lower extremity and comparison of this with more regional measures (calf) of perfusion (PET and PL) in a small cohort of individuals. TD measurement provides an estimate of total lower extremity FR, whereas PET and PL provided regional differences in the calf. This theoretically may have lead to an exaggeration or minimization of differences with TD, depending on the status of the circulation and the responsiveness of the various arterial beds of the lower extremity. PL did not provide any discriminative ability between the 2 groups of subjects and grossly underestimated FR measurements. A prior study that compared PET-derived flow at the level of the calf in subjects with PAD also showed very poor correlation between PL and PET (*4*). Potential explanations include the influence of slight movements and respiration on the acquired signal and variability in regional blood flow that may theoretically result in disproportionate expansion of regions in the calf, which may, in turn, result in insensitivity of PL to detect changes in flow at that level. TD and, to a certain extent, PET are protected from the variability because the area sampled is larger. Finally, in 2 of 5 PAD subjects, PET merely provided comparable values to that of TD. One could argue that because this occurred at the lower spectrum of flow ratios, this may not offer superior discrimination in a subset of subjects who are precisely the ones that may need such an assessment.

CONCLUSION

PET-derived calf FR assessment appears to generate sharper and more reproducible indices of peripheral FR compared with more conventional modalities of flow assessment and may have potential utility in the development of a simple noninvasive modality to assess responses to emerging therapies in atherosclerosis.

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